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NOTES ON SOME LEPIDOPTERA FROM EASTERN TEXAS [1]

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INTRODUCTION

In the latter part of 1922, and again in 1923, the writer was detailed to collect and rear Lepidoptera from eastern Texas, especially those of the Malvaceae. The project was carried out in connection with work on the eradication of the pink bollworm (*Pectinophora gossypiella* Saunders), under the supervision of the late W. D. Hunter, in charge. The main objects of the work were twofold; to discover, if possible, host plants of the pink bollworm other than cotton, and to enlarge the list of Lepidoptera likely to be mistaken for this comparatively new cotton pest.

Most of the collections were made in Liberty and Chambers Counties, in parts of which infestations of the pink bollworm had been found in 1917, 1919, and 1920. In 1918 and 1919 a similar study of the lepidopterous insects of malvaceous and related plants was made and the results published.[3] The pink bollworm was not found on any of the plants examined.

Of the 40 species of Lepidoptera listed in the present paper, four are described as new, and two, already described, are here recorded for the first time from the United States. One variety is also described as new, and one new genus has been erected by Busck. In the course of the work a large number of observations were made on rather well-known species. The present paper, however, has been confined practically altogether to new species and to previously unrecorded data regarding the habits and distribution of other forms.

FAMILY HESPERIIDAE

PYRGUS SYRICHTUS (FABRICIUS)

Papilio syrichtus Fabricius, 1775, Syst. Ent., p. 534.
Hesperia syrichtus Dyar, 1902, List N. Amer. Lepidop., no. 652.
Pyrgus syrichtus Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 493, 1917.

Larvae of this species were collected on leaves of the following malvaceous plants during 1923: *Sida rhombifolius* Linnaeus, at

[1] Received for publication May 12, 1926; issued November, 1926.
[2] The writer is indebted to Schaus, Busck, and Heinrich for determining the Lepidoptera and for other assistance; to Rohwer, Gahan, and Cushman for naming the Hymenoptera; and to Aldrich for identifying the dipterous parasites. He is also indebted to Bessey, of East Lansing, Mich., and Standley, of the National Herbarium, for determining the host plants. The drawings were made by H. B. Bradford, under the supervision of August Busck.
[3] HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807-836, illus. 1921.

Liberty, Tex., July 7; *Malvastrum americanum* (Linnaeus) Torrey, at Victoria, Tex., August 2 and August 25; and on the same host plants at Lake Charlotte, Tex., November 16. An adult emerged July 24, 1923, from a specimen taken at Liberty.

FAMILY SATURNIIDAE

AUTOMERIS IO (FABRICIUS)

Bombyx io Fabricus, 1775, Syst. Ent., p. 560.
Automeris io Dyar, 1902, List N. Amer. Lepidop. no. 753; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 780.

A colony of about 25 half-grown larvae of this moth was found defoliating a cotton plant at Liberty August 31, 1923. The larvae collected became mature during the last week of the month following and pupated within tough, oval-shaped, brownish cocoons. From these specimens several adults were reared during the latter half of April, 1924.

A single immature larva was also taken on cotton at Smith Point, Tex., October 4, 1923. Several days later two young larvae were collected on leaves of *Hibiscus lasiocarpus* Cavanilles at Liberty. A nearly full-grown larva was found feeding upon the leaflets of *Amorpha fruticosa* Linnaeus at Lake Charlotte, October 23, 1923.

FAMILY NOCTUIDAE

SUBFAMILY AGROTINAE

HELIOTHIS OBSOLETA (FABRICIUS)

Bombyx obsoleta Fabricius, 1793, Ent. Syst., 3 (1): 456.
Chloridea obsoleta Hampson, 1903, Cat. Lepidop. Phal. Brit. Mus. 4: 45, 657.
Heliothis obsoleta Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 1090.

Larvae of this species, which is known as the "cotton bollworm," were collected in maturing bolls of *Hibiscus lasiocarpus* Cavanilles and *H. militaris* Cavanilles at Lake Charlotte from August 18 to September 8, 1922, and in the bolls and open flowers of *H. lasiocarpus* at Liberty during September of the same year. Pupation took place within oval-shaped cells in the soil, the average pupation period of the five adults reared being 11 days. The adults emerged from September 11 to October 13, 1923.

Larvae were also found feeding upon leaves and bolls of okra (*Hibiscus esculentus* Linnaeus) at Smith Point October 3, 1923. One adult emerged October 30, 1923, and two emerged April 8 and April 17, 1924.

HELIOTHIS VIRESCENS (FABRICIUS)

Noctua virescens Fabricius, 1781, Spec. Ins. 2: 216.
Chloridea virescens Dyar, 1902, List N. Amer. Lepidop., no. 2296.
Heliothis virescens Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 1091.

Larvae were collected on leaves and bolls of okra (*Hibiscus esculentus* Linnaeus) at Smith Point October 3, 1923. The larvae pupated the same month, and from these specimens three adults were reared during the first part of the following April.

SUBFAMILY ERASTRIINAE

XANTHOPTERA NIGROFIMBRIA GUENÉE

Xanthoptera nigrofimbria Guenée, 1852, Spec. Gén. 6: 241.

One larva was found in a ripening capsule of *Ipomoea speciosa* Walter at Smith Point, September 1, 1922. Pupation took place about September 3 within a thin cocoon fastened to the stem of the plant and covered with small pieces of leaf. The moth emerged September 15, 1922.

HELIOCONTIA MARGANA (FABRICIUS)

Paralis margana Fabricius, 1794, Ent. Syst., 3 (2): 257.
Spragueia sordida Dyar, 1902, List N. Amer. Lepidop., no. 2710.
Heliocontia margana Hampson, 1910, Cat. Lepidop. Phal. Brit. Mus. 10: 666–667; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 2924.

Larvae of this species were collected on the leaves and buds of *Malvastrum americanum* (Linnaeus) Torrey at Victoria August 21, 1923. From this material a male emerged September 3 of the same year.

SUBFAMILY ACONTIINAE

BAGISARA RECTIFASCIA (GROTE)

Schinia rectifascia Grote, 1874, Boston Soc. Nat. Hist. Proc. 16: 242.
Atethmia rectifascia Dyar, 1902, List N. Amer. Lepidop., no. 2267.
Bagisara rectifascia Hampson, 1910, Cat. Lepidop. Phal. Brit. Mus. 9: 156; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 2730; Heinrich, 1921, Jour. Agr. Research 20: 834.

Larvae were collected on leaves of *Hibiscus lasiocarpus* (?) at Smith Point August 10, 1922. One specimen pupated August 13 in a loosely folded leaf, and the adult emerged 11 days later. An adult was reared November 7, 1923, from larvae collected on leaves of *Malvaviscus drummondii* Torrey and Gray at the same locality October 3, 1923.

The larvae were found in large numbers on Malvaviscus at Lake Charlotte during October, 1923. When full grown the larva enters the soil and constructs a flattened circular cell which is lined with silk, and in which it rests until it pupates the following spring. No adults were obtained from these larvae.

The full-grown larva is 30 to 35 mm. long, 2 to 2½ mm. wide, and nearly cylindrical. The body above is light green, with seven narrow, wavy stripes of darker green, one dorsal, two subdorsal on each side, and one lateral; paler beneath. Head pale greenish white with four transverse broken rows of grayish spots; thoracic shield and anal plate of body color; thoracic legs pale; abdominal legs pale, rather elongate, one pair each on fifth and sixth abdominal segments; (absent on third and fourth abdominal segments); crochets about 20 in number, arranged in a semicircle on inner side; anal legs pale, well developed; anal fork lacking; body setae elongate and slender. The dorsal surface of the full-grown larva is often tinged with a pale wine color.

Heinrich has advised the writer that this species should be placed in the Acontiinae rather than in the Acronyctinae because of characters of the larva and the adult, the latter having vein 5 of the hind wing well developed.

Larvae collected at Lake Charlotte were found to be parasitized by *Meteorus laphygmae* Viereck (Hymenoptera). Two of these emerged about December 1, 1923, from oval-shaped cocoons spun by the parasitic larvae after the latter had left the body of the host.

SUBFAMILY PHYTOMETRINAE

PHYTOMETRA OO (CRAMER)

[*Phalaena*] *oo* Cramer, 1782, Pap. Exot. 4: 45, pl. 311, fig. E (nec. F).
Phytometra oo Hampson, 1913, Cat. Lepidop. Phal. Brit. Mus. 13: 522.

A single adult was reared about December 1, 1923, from a larva found in a fold of an okra leaf at Smith Point November 1, 1923. The larva was a very pale green, except for a large blackish area on the middle of the dorsal surface. The dark area was present on the pupa and is retained on the pupal case.

SUBFAMILY EREBINAE

ANOMIS EROSA HÜBNER

Anomis erosa Hübner, 1818, Zutr. Samml. Exot. Schmett., p. 19, fig. 287, 288; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3407.

Larvae and pupae were collected in large numbers on okra at Smith Point during October, 1923. The larvae feed upon the leaves and pupate in a long roll or fold in the leaf. Emergence dates ranged from October 8 to about December 1, 1923.

From this material four adults of the hymenopterous parasite *Itoplectis conquisitor* (Say) were reared during December, 1923, each parasite developing singly within the body of its host and emerging during the pupal stage of the latter.

One larva of *Anomis erosa* was taken on *Malvaviscus drummondii* Torrey and Gray at Lake Charlotte October 23, 1923. The specimen pupated one week later in a loosely tied leaf, and about December 1 of the same year a dipteron, *Zenillia blanda* Osten Sacken, emerged from the pupa.

A series of adults of the hymenopteron *Syntomosphyrum esurus* Riley emerged from a pupa of *Anomis erosa* during the first part of December, 1923. A single emergence hole in the dorsal surface of the abdomen of the host was made. This parasite was also reared in large numbers during the winter of 1923–24 from pupae of *Alabama argillacea* (Hübner), taken on cotton in various parts of Liberty and Chambers Counties.

ANOMIS EXACTA HÜBNER

Anomis exacta Hübner, 1810, Samml. Exot. Schmett., v. 2, pl. 411; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3410.

One larva was collected on Malvaviscus at Lake Charlotte October 12, 1923. The specimen pupated October 17 in a loosely tied leaf of the host plant, and the adult emerged November 8, 1923.

FAMILY GEOMETRIDAE

SYNCHLORA DENTICULARIA (WALKER)

Nemoria (?) denticularia Walker, 1861, List Lepidop. Brit. Mus. 22: 536.
Synchlora excurvaria Hulst, 1895, Ent. News 6: 71.
Synchlora denticulata Dyar, 1902, List N. Amer. Lepidop., no. 3580.
Synchlora denticularia Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 3801.

Four adults were reared June 30, to July 16, 1923, from pupae collected on the flower heads of *Rudbeckia maxima* Nuttall at Liberty June 27 and July 7, 1923. One larva was taken on the flower head of *Helianthus* sp. at Smith Point October 2, and a second on *Malvastrum americanum* (Linnaeus) Torrey at Lake Charlotte November 16, 1923. An adult was reared October 20, 1923, from the first specimen. The larvae are surface feeders, and carry about with them seeds and particles of the host plant attached to the spiny tubercles on the dorsal surface of the abdominal segments. Pupation takes place within a very frail web covered with small pieces of the host plant.

FAMILY PYRALIDAE

SUBFAMILY PYRAUSTINAE

LOXOSTEGE HELVIALIS (WALKER)

Spilodes helvialis Walker, 1859, List Lepidop. Brit. Mus. 18: 772.
Loxostege helvialis Dyar, 1902, List N. Amer. Lepidop., no. 4351; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5022.

Several larvae of this species were found feeding within loosely rolled leaves of *Amaranthus spinosus* Linnaeus, at Liberty July 2 to 4, 1923. On July 4 one larva was also taken in a rolled leaf of *Sida rhombifolia* Linnaeus, in close proximity to some of the Amaranthus plants. Feeding of the larva on Sida was not noted.

The feeding larva is pale green, and when full grown is about 18 mm. in length. Just before pupation its dorsal surface becomes either a solid deep pink or is marked with broad stripes of dark pink. Moths emerged July 11 and July 16, 1923, from the tough, dark brown cocoons, to the outside of which leaves of the host plant had been fastened. From a whitish cocoon spun by one of the larvae a dipteron emerged July 18, but it was in poor condition and could not be determined.

PYRAUSTA PHOENICEALIS (HÜBNER)

Haematia phoenicealis Hübner, 1818, Zutr. Exot. Schmett., fig. 115, 116.
Pyrausta phoenicealis Dyar, 1902, List N. Amer. Lepidop., no. 4448; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5145.

Larvae of this species were collected in frail silken tubes in the flower heads of a mint, *Mesosphaerum rugosum* (Linnaeus) Pollard, at Liberty, during September and at Smith Point on October 2, 1923. The full-grown larva is about 15 mm. long, pale green, with pale pinkish-purple stripes, and with conspicuous, black, elongate-oval chitinizations at the base of the body setae. Adults were reared September 30, 1923, and from February to April, 1924. The species overwinters as full-grown larvae.

NOCTUELIA RUFOFASCIALIS (STEPHENS)

Ennychia rufofascialis Stephens, 1834, Illus. Brit. Ent. Haust. 4: 33.
Noctuelia rufofascialis Barnes and McDunnough, 1918, Contrib. Nat. Hist. Lepidop. N. Amer. 4: 167; Heinrich, 1921, Jour. Agr. Research 20: 829–830.

Heinrich has recently received larvae of this species collected at Eagle Pass, Tex., by C. E. Bellis in cotton shipped from Rosita, Tex. This is a new host record. The insect has not been taken in the eastern part of Texas.

SUBFAMILY THYRIDINAE

MESKEA DYSPTERARIA GROTE

Meskea dyspteraria Grote, 1877, Canad. Ent. 9: 115; Dyar, 1902, List N. Amer. Lepidop, no. 4139; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 4897; Heinrich, 1921, Jour. Agr. Research 20: 828–829.

Galls in the stems of *Malvaviscus drummondii* Torrey and Gray containing larvae of this species were collected at Lake Charlotte September 21 and October 27, 1922, and October 23 and November 16, 1923. The galls were found mainly on small, stunted plants which made up one large colony. Other near-by colonies of the same plant, but of normal growth, were not at all infested. A few galls were found on Malvaviscus at Liberty, during September, 1923. They were usually formed singly on the upper half of the main stem, but occasional plants bore galls on the branches. In one case a very elongate enlargement on a branch of the host contained three larvae of *Meskea dyspteraria*, each in a separate cavity in the stem.

The conspicuous galls are of a light russet color, elongate-oval in shape, and usually quite regular in outline. They range in length from 20 to 50 mm., and in diameter at the middle or largest part of the gall from 6 to 13 mm., the average being about 40 mm. long and 11 mm. wide. In the upper part of the gall is a small round hole scarcely 1 mm. in diameter, through which frass in the form of short cylindrical pellets was discharged at regular intervals for a short period after the specimens were collected. Immediately after each operation the larva spun a web over the hole on the inside of the burrow. The burrow is lined with a thin web and is kept quite clean.

The winter is passed in the larval stage. When ready to pupate the larva cuts a round hole at the lower extremity of the cavity through the woody part of the gall to the outer surface, leaving the thin bark or epidermis unbroken. Pupation then takes place within the cavity, the caudal end of the pupa being fastened to the upper part of the burrow and the cephalic end pointing toward the newly constructed hole at the base of the cavity, through which the adult escapes upon emergence. The pupal case remains entirely concealed within the gall. Pupation took place during the latter part of March and the first part of April. From larvae collected September 21 and October 27, 1922, adults were reared April 17 to April 30, 1923, and from specimens collected October 23 and November 16, 1923, moths emerged April 13 to April 29, 1924.

Upon emergence the sluggish adults climbed to the upper part of the stems of the host plant in the rearing cages and rested in a very characteristic pose. In this pose the fore wings are more or less

folded along the anterior margin of the hind wings, which are spread, and in this position both pairs of wings are moved around toward the ventral surface of the insect until they form an acute angle with each other, but remain nearly at right angles to the thorax. Both sexes rest with the abdomen curved slightly upward.

Three specimens of the hymenopterous parasite *Calliephialtes grapholithae* (Cresson) were reared from larvae of *Meskea dyspteraria* Grote, each parasite developing singly within the body of its host and emerging through a small hole in one end of the gall. One specimen emerged on February 18 and two on March 12, 1924, from larvae collected at Lake Charlotte during November, 1923.

SUBFAMILY PHYCITINAE

HOMOEOSOMA ELECTELLUM (HULST)

Anerastia electella Hulst, 1887, Entomologica Americana 3: 137–138.
Homoeosoma electellum Hulst *in* Dyar, 1902, List N. Amer. Lepidop., no. 4865; Heinrich, 1921, Jour. Agr. Research 20: 832.

Two adults were reared September 11 and October 5, 1922, from larvae found feeding in the flower heads of *Helianthus* sp. at Smith Point August 9 and September 14. From larvae collected in the flower heads of *Rudbeckia maxima* Nuttall and *Helianthus* sp. at Liberty in June and July, 1923, adults were reared June 27 to July 16 of the same year. Pupation took place in the irregular webbed channels made by the larvae.

From a parasitized larva taken in a flower head of *Rudbeckia maxima* a specimen of *Spilochalcis delira* (Cresson) was reared July 5, 1923.

EUZOPHERA SEMIFUNERALIS (WALKER)

Nephopteryx semifuneralis Walker, 1863, List Lepidop. Brit. Mus. 27: 57.
Euzophera semifuneralis Hulst, 1890, Trans. Amer. Ent. Soc., 17: 175; Dyar, 1902, List N. Amer. Lepidop., no 4832; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5720.

Four adults were reared March 18 to April 14, 1924, from larvae collected in stalks of corn at Snyder, Scurry County, December 19, 1923. The larvae construct rather heavy webs in the irregular burrows in the pith of the host for pupation.

EPHESTIA CAUTELLA (WALKER)

Pempelia cautella Walker, 1863, List Lepidop. Brit. Mus. 27: 73.
Ephestia cautella Rebel, 1901, Cat. Lep. Eur. 2, no 271; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 5801.

Adults of this species were reared from larvae found feeding upon the pulp of ripened bolls of *Malvaviscus drummondii* Torrey and Gray collected at Lake Charlotte during August, 1922. The larvae pupated within folds in small pieces of cloth in the rearing cages. Emergence dates ranged from October 7 to October 17, 1922, the pupation period averaging 10 days.

FAMILY COSMOPTERYGIDAE

CHAETOCAMPA BUSCK,[4] NEW GENUS

(Fig. 1, A to F; fig. 2, D; fig. 3, A, B, C)

Type.—*Chaetocampa crotonella* Bottimer, hereinafter described.

Chaetocampa, n. g.

Labial palpi curved upward, sickle shaped, smooth, reaching vertex, terminal joint nearly as long as second and pointed. Antennae with strong pecten on basal joint. Face and head smooth. Fore wings narrow lanceolate; 12 veins; 6 and 7 long-stalked, inclosing apex; 8 out of their stalk; 5 out of base of stalk; rest separate; 1b not furcate at base; 1c present, strong. Hind wings half as wide as fore wings; lanceolate; 8 veins; 6 and 7 very long-stalked or united; rest separate; 5 nearest 6 and 7. A series of long spinelike scales along costal edge. Posterior tibiae strongly tufted above. Male genitalia with rounded broad uncus; gnathos divided into two free knobbed arms; socii absent; tegumen slender, elongate; harpes greatly reduced, small, semicircular; vinculum narrow, with long anteriorly projecting process; annellus strongly developed, with chitinous processes; aedoeagus long, slender, and nearly straight. Larva with secondary setae.

The genus is for the present placed in the family Cosmopterygidae, where it would go on its oral and pterogostic characters, but the larvae and the genitalia prove that this family is heterogeneous and must be divided. The present genus does not go with Cosmopteryx and its allies, but represents a distinct family, characterized by the quite different genitalia and by the hairy larvae. (Fig. 3, C)

Belonging to this family and closely allied to the present genus is the Transcaucasian genus Parametriotes Kusnezov (type, *P. theae* Kusnezov),[5] and some of the species at present wrongly referred to the genus Batrachedra Stainton.

The definition of the family is advisedly postponed until the entire group can be properly worked up.

CHAETOCAMPA CROTONELLA, NEW SPECIES

Chaetocampa crotonella, n. sp.

Antenna above whitish ochreous; beneath black, pubescent, and with whitish ochreous scales at apex of each joint, giving under surface a serrate appearance. Labial palpus whitish ochreous; second joint with a few dark fuscous scales on upper and outer sides, especially near apex; terminal joint with a few dark fuscous scales in middle on outer side. Face, head, and thorax whitish ochreous. Fore wing whitish ochreous, overspread with dark fuscous scales, more strongly above and beyond cell; a distinct black dot at end of cell; from there an indistinct longitudinal dark streak to apex, and a more or less distinct marginal series of black dots; cilia light ochreous fuscous. Hind wing dark silvery fuscous; cilia light ochreous. Under surface of both fore and hind wings dark silvery fuscous. Abdomen whitish ochreous. Legs whitish ochreous, shaded with light fuscous exteriorly.

Male genitalia of paratype from Smith Point figured (fig. 1, D, E).

Alar expanse.—9 to 13½ mm.

Type.—Cat. No. 27333, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Croton engelmannii* Ferguson.

Described from male type (May 27, 1924) and three male and four female paratypes reared April 9 to June 16, 1924, from larvae collected in seeds October 19, 1923; one male and two female paratypes

[4] Description of the new genus furnished by August Busck.

[5] KUSNEZOV, N. J. DESCRIPTION OF PARAMETRIOTES THEAE, GEN. N., SP. N. (LEPIDOPTERA, TINEIDAE), A NEW ENEMY OF THE TEA BUSH IN TRANSCAUCASIA. Russ. Ent. Obozr. (Rev. Russe Ent.) 15: 627–652, illus. 1916. [In Russian. English summary, p. 642–649.]

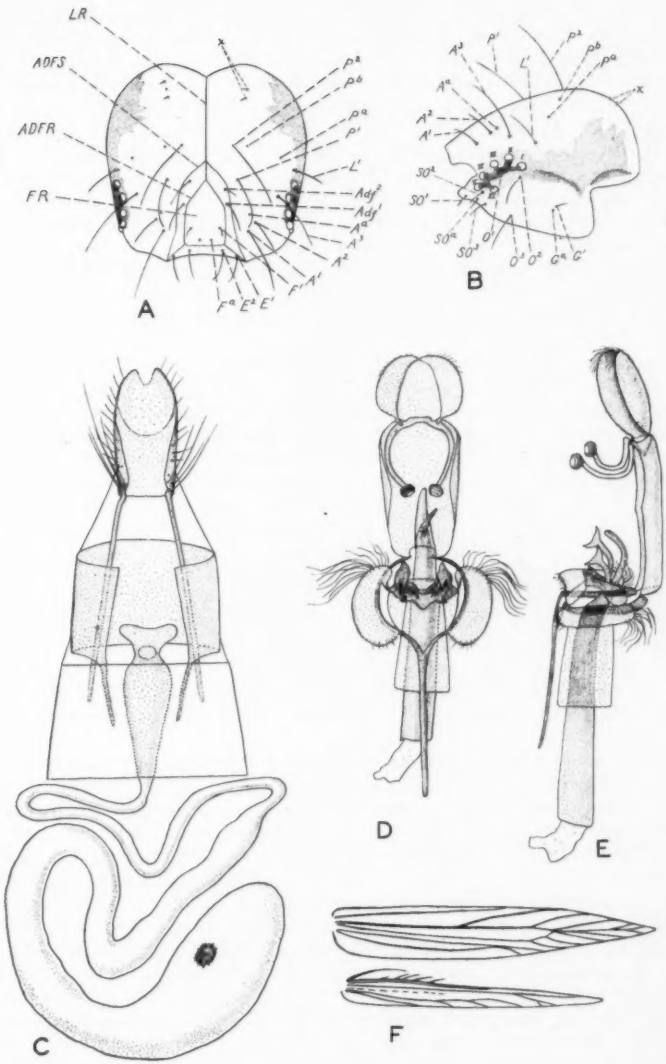

FIG. 1.—*Chaetocampa crotonella:* A, head of larva, front view; B, head of larva, side view; C, female genitalia; D, male genitalia, front view; E, male genitalia, side view; F, wing venation

A^1, A^2, A^3, A^a, anterior setae and puncture of epicranium; Adf^1, Adf^2, adfrontal setae and puncture of epicranium; ADFR, adfrontal ridge of frons; ADFS, adfrontal suture; E^1, E^2, epistomal setae; F^1, F^a, frontal seta and puncture; FR, frons; G^1, G^a, genal seta and puncture of epicranium; L^1, lateral seta and puncture of epicranium; LR, longitudinal ridge of frons; O^1, O^2, O^3, ocellar setae and puncture of epicranium; P^1, P^2, P^a, P^b, posterior setae and punctures of epicranium; SO^1, SO^2, SO^3, SO^a, subocellar setae and puncture of epicranium; X, ultraposterior setae and punctures of epicranium.

reared July 28, 1923, from larvae collected in leaf galls July 6, 1923, all from the type locality; and one male paratype reared August 31, 1922, from a leaf gall found at Smith Point, Tex.

Chaetocampa crotonella is quite common in parts of Liberty and Chambers Counties. Larvae were collected in galls in the petioles of leaves of the host plant from June 28 to August 10. The galls are usually formed next to the leaf blade and are somewhat irregularly conical in shape, tapering away from the base of the leaf blade. The enlargements average from 3 to 4 mm. in diameter at the widest point and range from 5 to 15 mm. in length.

Later in the season the larvae are to be found only in the immature three-seeded capsules of the same plant. Larvae were noted in the seeds as early as July 11, and were collected as late as November 13 in late-maturing plants. In the capsule the larva feeds upon the contents of the seeds, going from one to another through very clean-cut holes.

The larva possesses secondary body setae. The younger stages are white. When full grown the larva is 8 to 10 mm. long and purple-black, the color becoming paler toward the anterior end; thoracic segments each with a middle lateral spot of the darker color. Head pale purplish white, with lateral margins purple-black; thoracic shield purplish white with darker spots, posterior margin broadly purple-black; thoracic legs pale; abdominal legs normal, crochets (fig. 2, D) about 15 in number, arranged in a circle broken outwardly; anal plate of body color; anal fork absent.

Pupation of the larva found in the galls takes place within the enlarged petioles of the leaf, the pupal case remaining in the gall after the emergence of the moth. When full grown the seed-feeding larva leaves the capsule of the host and seeks a suitable place in which to pupate, but does not enter the soil. Those in the rearing cages pupated in pieces of cork, the cases remaining in the silk-lined cavities upon emergence of the adults. The species overwinters as pupae.

The pupa (fig. 3, A, B) is 5 to 6½ mm. long and 1 to 1¼ mm. wide; nearly cylindrical, slightly dorsally concave; color yellowish brown; surface granular, dorsal surface of abdominal segments finely transversely corrugated. Front, vertex, prothorax, and anterior half of mesothorax thickly covered with very short, stout spines, and with a few larger scattered spines; a large curved spine at lower margin of eye, several on first joints of antennae, one on outer hind angle of vertex, several on posterior half of mesothorax, and one near outer margin of metathorax. Maxillae and prothoracic legs about half as long as wing cases; antennae, metathoracic legs, and wing cases reaching beyond fifth abdominal segment; serrations of antennae plainly visible; antennae meeting at fourth abdominal segment and diverging posteriorly, exposing the tips of metathoracic legs. Abdomen with movable joints between segments 5 and 6, and 6 and 7, the sutures between these segments being wide and deep; caudal end bluntly pointed; segments 9 and 10 fused ventrally; anal prolegs with crochets present.

Larvae found in the galls produced moths July 13 to August 31 of the same year. A few adults were reared during the later part of September from larvae taken in seeds, but most of the seed-feeders did not produce moths until the following year, the emergence dates

ranging from April 5 to June 17. The adult rests with all its legs touching the surface, with the head slightly elevated, and with the antennae above the wings along the side of the body.

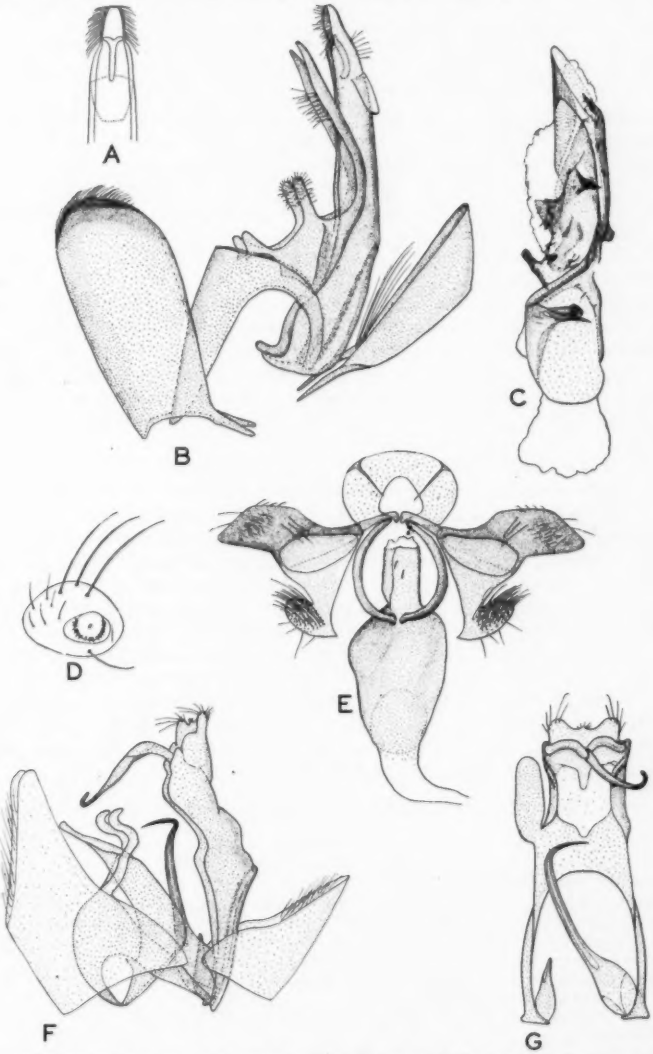

FIG. 2.—*Gelechia monotaeniella:* A, male genitalia, uncus; B, male genitalia, aedoeagus removed; C, male genitalia, aedoeagus. *Chaetocampa crotonella:* D, abdominal proleg of larva. *Isophrictis similiella:* E, male genitalia. *Recurvaria eryngiella:* F, male genitalia, side view; G, male genitalia, front view

Two specimens of *Bassus gibbosus* Say (Hymenoptera) were reared January 14, 1924, from Croton seed capsules collected at Liberty and are probably parasitic upon the lepidopteron.

FAMILY GELECHIIDAE

ISOPHRICTIS SIMILIELLA (CHAMBERS)

(Fig. 2, E; fig. 3, D, E, F)

Gelechia similiella Chambers, 1872, Canad. Ent. 4: 193.
Paltodora similiella Busck, 1903, U. S. Natl. Mus. Proc. 25: 779–780.
Isophrictis similiella Meyrick, 1917, Ent. Mo. Mag. 53: 113; Heinrich (in part), 1921, Jour. Agr. Research 20: 813–814.

In Heinrich's paper two species of Isophrictis are confused under the name *similiella*—the true *similiella* (Chambers) and *rudbeckiella*, a species here described as new, the genitalia figured in Plate 95, A, of his paper belonging to the new species.[6] Both species have the harpe of the male genitalia divided, but in the new species it is nearly twice as long as in *similiella*.

Five adults were reared from September 26 to October 21, 1922, from larvae collected in the flower heads of a tall wild sunflower (*Helianthus* sp.) at Smith Point in September. The larvae were found in large numbers in the dried flower heads of a similar sunflower at Sweetwater and San Angelo, in northern Texas, during December. Specimens collected at the latter locality December 14, 1923, remained over winter as larvae. In the rearing cage pupation took place from May to June, the adults emerging from May 7 to July 1, 1924.

The larvae range from 5 to 10 mm. in length and have three or four crochets on the anal and abdominal legs. The pupa (fig. 3, D, E, F) is 5 to 6½ mm. long, 1¼ to 1½ mm. wide; cephalic end pubescent; wing cases reaching nearly to eighth abdominal segment; tips of metathoracic legs exposed beyond tips of wing cases; maxillae extending well beyond caudal end, which is armed with numerous elongate hooked spines and a dorsally projecting cremaster.

From the San Angelo material the following Hymenoptera were reared during April, 1924: *Microbracon mellitor* (Say), one specimen; *M. nuperus* (Cresson), one specimen; *Microbracon* n. sp., one specimen; *Zaglyptonotus schwarzi* Crawford, three specimens; *Callimome* sp., three specimens; and four specimens of a pteroma id. Six adults of *Cremastus facilis* (Cresson) were also obtained, the emergence dates ranging from April 27 to July 14, 1924.

ISOPHRICTIS SIMILIELLA DENOTATA, NEW VARIETY

(Fig. 3, G, H)

Isophrictis similiella denotata, n. v.

Differs from the true *similiella* in its darker color, and in having the fore wings covered with white-tipped black scales without the distinct golden longitudinal streaks. These are replaced by scattered reddish brown scales, which at the apex form a V-shaped mark as in the typical specimens.

The genitalia are the same as in *similiella*.

Alar expanse.—9 to 16 mm.

Type.—Cat. No. 27334, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Helianthus mollis* Lamarck.

Described from male type and 4 male and 11 female paratypes reared September 17 to October 24, 1923, from larvae collected during the first part of the same month.

[6] HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807–836, pl. 95, A. 1921.

The habits of the larva are similar to those of the larva *Isophrictis similiella*. The larva differs from that of the true *similiella* in having one crochet, rarely two, on the anal and abdominal legs. The pupa

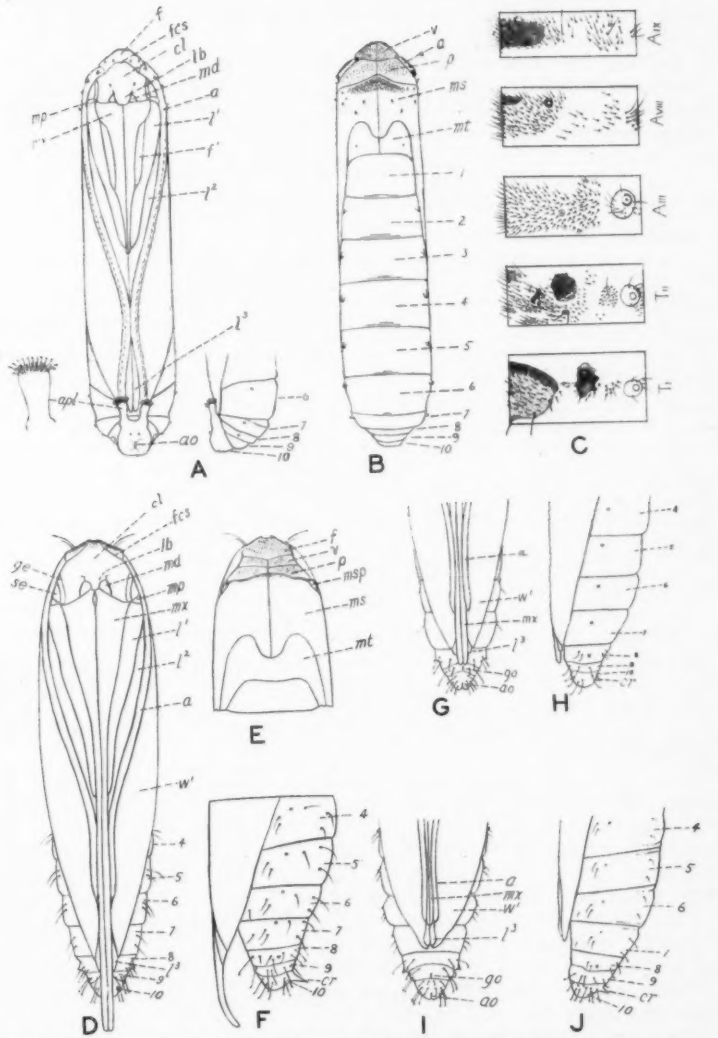

FIG. 3.—*Chaetocampa crotonella:* A, pupa, front and side views; B, pupa, back view; C, setal map of larva. *Isophrictis similiella:* D, pupa, front view; E, pupa, back view; F, pupa, side view. *Isophrictis similiella denotata:* G, posterior tip of pupa, front view; H, posterior tip of pupa, side view. *Isophrictis rudbeckiella:* I, posterior tip of pupa, front view; J, posterior tip of pupa, side view

a, antenna; *ao*, anal opening; *apl*, anal proleg; *cl*, clypeus; *cr*, cremaster; *f*, front; f^1, femora of prothoracic leg; *fcs*, fronto-clypeal suture; *ge*, glazed eye; *go*, genital opening; l^1, prothoracic leg; l^2, mesothoracic leg; l^3, metathoracic leg; *lb*, labrum; *md*, mandible; *mp*, maxillary palpus; *ms*, mesonotum; *msp*, mesothoracic spiracle; *mt*, metanotum; *mx*, maxilla; *p*, pronotum; *se*, sculptured eyepiece; *v*, vertex; w^1, mesothoracic wing

(fig. 3, G, H) differs in the length of the maxillae, which extend only slightly beyond the tips of the wing cases and metathoracic legs; in none do they reach the tip of the abdomen.

Twelve specimens of *Tachinophyto* sp. (Diptera) were reared September 18 to October 24, 1923, as parasites of the larvae of this species.

During the latter half of September, 1923, the following Hymenoptera were reared from the Liberty material: *Apanteles dakotae* Muesebeck, four specimens; *Callimome* sp., one specimen; and *Cremastus* sp., one specimen.

ISOPHRICTIS RUDBECKIELLA, NEW SPECIES

(Fig. 3, I, J)

Isophrictis rudbeckiella, n. sp.

Antenna with basal joints whitish, overlaid with fuscous scaling; apically becoming golden ochreous, strongly annulated with black, the ochreous scales spreading, giving a serrated appearance, especially on apical half; finely pubescent beneath. Palpus white; second joint with outer upper edge fuscous, and with outer side and inner apical third of well-developed brush fuscous; terminal joint with outer side fuscous, extreme tip black. Face white. Head and thorax covered with white-tipped dark scales. Fore wing covered with white-tipped dark scales, the darker color fuscous at base, becoming black at apex of wing; marked with narrow longitudinal streaks of white-tipped golden scales, one below costa, one along fold, and a third midway between the two, rather distinct on basal half, the streaks beyond middle becoming shorter, more numerous, and confused, forming a rather distinct V-shaped area of golden scales pointing toward the apex just before a strong edging of white-tipped black scales; cilia light fuscous, at apex darker, and crossed by two nearly black lines. Hind wing dark silvery fuscous; cilia light fuscous. Both fore and hind wings dark fuscous beneath. Abdomen dark silvery fuscous, with silvery lateral streak. Legs silvery white, heavily overlaid with fuscous exteriorly.

Alar expanse.—11½ to 16 mm.

Type.—Cat. No. 27335, United States National Museum.

Type locality.—Liberty, Tex.

Food plant.—*Rudbeckia maxima* Nuttall. Also reared from *R. hirta* Linnaeus.

Described from male type and four male and six female paratypes reared June 10 to June 28, 1924, from larvae collected in flower heads of *Rudbeckia maxima* at Liberty September, 1923, and one male paratype reared from *Rudbeckia* sp. (Heinrich, May 26, 1918) at Alvin, Brazoria County.

This species is distinguished from *similiella* Chambers only by means of the male genitalia, slight but constant larval and pupal characters, and host plants. The male genitalia of *rudbeckiella* are figured by Heinrich[7] under the name *Isophrictis similiella* Chambers.

The larva feeds throughout the flower head of its host and pupates within the burrow or in the upper part of the stem. From that of *similiella* the larva differs in having one or two crochets on the anal legs and none on the abdominal legs. The pupa (fig. 3, I, J) differs in having the maxillae considerably shorter than the wing cases, which are equal in length to the metathoracic legs and extend slightly beyond the seventh abdominal segment.

Two generations were noted. Larvae and pupae collected in *Rudbeckia maxima* on July 17, 1922, produced adults from July 26 to August 12, 1922, and larvae taken in the same host in September,

[7] HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 807-836, pl. 95, A. 1921.

1923, produced moths the following June. A large series of the second generation was reared during June, 1924, from flower heads of *Rudbeckia hirta* L nnaeus taken at Liberty in November of the previous year. The winter is passed in the larval stage.

The larvae were found to be parasitized by several Hymenoptera. From those in *Rudbeckia maxima* three specimens of *Aerophilus* n. sp. were reared April 3, April 8, and June 11, 1924, and one specimen of *Cremastus* sp. emerged March 26 of the same year. Sixteen specimens of *Aerophilus* n. sp. (March 31 to June 19), one specimen of *Microbracon* n. sp. (May 13), and two specimens of *Cremastus minor* Cushman (April 30) were obtained during 1924 from the larvae in *R. hirta*.

The species recorded by Braun[8] as *Isophrictis similiella* Chambers is presumably referable to the present species.

ARISTOTELIA CORALLINA WALSINGHAM

Aristotelia corallina Walsingham, 1909, Biol. Centr.-Amer., Lep.-Het. 4: 23.

Larvae of this species were collected on leaves of *Vachellia farnesiana* (Linnaeus) Wight and Arnot at Lake Charlotte, in September and October, 1923. From this material a series of 12 adults emerged October 4 to October 13, 1923, and January 11 to January 12 of the following year. This is the first record of this Mexican species occurring in the United States.

The larvae construct silken tubes, to which are fastened leaflets of the host plant. The full-grown larva is 8 to 10 mm. long. Body with second and third thoracic segments broadly banded dorsally, with purplish black, pale lavender beneath, intersegmental areas white; first abdominal segment with anterior half white, posterior half purplish black; second to fourth abdominal segments with posterior dorsal third purplish black, anterior dorsal third same color but marked with broad wavy white bands, middle dorsal third pale lavender with similar white markings; bands of first to fourth abdominal segments becoming paler beneath; fifth to tenth abdominal segments beneath pale brownish, more or less mottled with white, dorsally purplish black except for narrow dorsal whitish stripe from anterior margin of sixth abdominal segment to caudal end; a similar broad whitish lateral stripe extending from just before middle of fifth abdominal segment to caudal end; the three whitish bands mottled with pale lavender, becoming pale brown on last segment. Thoracic legs black; abdominal legs slightly elongate, crochets numerous, arranged in a complete circle; anal plate with lateral margins broadly brownish black, middle stripe whitish; anal fork well developed, two-pronged, black.

The pupa is 4 to 4½ mm. long, about 1 mm. wide; dark reddish brown; entirely pubescent; antennae, metathoracic legs, and wing cases reaching nearly to posterior margin of fifth abdominal segment; caudal end with numerous long, hooked spines, and cremaster.

[8] BRAUN, A. F. NOTES ON MICROLEPIDOPTERA WITH DESCRIPTIONS OF NEW SPECIES. Ent. News 32:8. 1921.

LATHONTOGENUS PALPIGERA WALSINGHAM

Gelechia palpigera Walsingham, 1891, Ent. Soc. Lond. Trans., p. 94, pl. 4, fig. 31.
Lathontogenus adustipennis Walsingham, 1897, Zool. Soc. Lond. Proc. 1: 88.
Paraspistes ioloncha Meyrick, 1905, Jour. Bombay Nat. Hist. Soc. 16: 600; 1911, Linn. Soc. Lond., Trans. (2), 14: 274.
Lipatia crotalariella Busck, 1910, Trinidad Dept. Agr. Bul. 9: 243, 244.
Paraspistis palpigera Busck, 1914, U. S. Natl. Mus. Proc. 47: 10, 11.
Lathontogenus palpigera Walsingham, 1915, Biol. Centr.-Amer., Lep.-Het. 4: 409.

A single adult was reared March 9, 1923, from pods of *Vachellia farnesiana* (Linnaeus) Wight and Arnot collected at Kingsville during January, 1923. The species is known from the Tropics, and has not heretofore been recorded from the United States.

RECURVARIA ERYNGIELLA, NEW SPECIES

(Fig. 2, F, G)

Recurvaria eryngiella, n. sp.

Antenna golden ochreous, basal joint white with small black spot above. Labial palpus white with dark fuscous scales on outer side at base; terminal joint with two broad black annulations, one near base and the other near tip. Face and head white. Thorax white with base of patagium and posterior dot black. Forewing white, strongly overlaid on outer two-thirds with golden ochreous scales, especially below the black costal markings; costal edge with small black streak at base, narrowly black to the middle, from which a short, outwardly oblique, black streak runs toward a few black scales on end of cell; at apical third a similar smaller, outwardly oblique, black streak; a few scattered black scales at apex; a black spot below middle of fold, and a smaller black dot near end of dorsum; cilia golden white, with scattered black dots near apex. Hind wing light silvery fuscous; cilia white. Abdomen and a strong hair pencil at base of hind wing golden ochreous. Legs golden ochreous, with broad black bars and annulations, the darker markings less pronounced on the hind legs.

Male genitalia of type figured (fig. 2, F, G).

Alar expanse.—7 to 10 mm.

Type.—Cat. No. 27336, United States National Museum.

Type locality.—Stowell, Chambers County, Tex.

Food plant.—*Eryngium aquaticum* Linnaeus.

Described from male type and 17 male and 34 female paratypes reared July 11 to July 16, 1923, from larvae and pupae collected July 3, 1923.

This species is near *variella* Chambers and *apicitripunctella* Clemens. From the former it differs in having the labial palpi and the legs marked with black. From the latter it is distinct in having the face, head, and thorax white, and in lacking the annulations on the antennae.

As many as 10 larvae were removed from a single flower head in which feeding had taken place. Pupation occurs within the silk-lined channels made by the larvae, the pupal cases remaining in the cavities after emergence of the adults. The full-grown larva is 7 to 8 mm. long; body color white, each segment with a broad, deep pink band on posterior half of dorsal surface, the white of the anterior half of thoracic segments 2 and 3 and abdominal segments 1 to 4 quite distinct, but that of the other abdominal segments becoming narrower and less distinct posteriorly; under surface similarly banded but paler. Head pale yellowish brown; thoracic shield slightly lighter in color, usually with a darker lateral border on each side; chitinizations at base of body setae small, round, yellowish in color; thoracic legs yellowish; crochets of abdominal legs numerous, arranged in a circle broken inwardly; anal plate yellowish brown; anal fork well developed, eight-pronged.

The pupa is about 4 mm. in length and 1 mm. in width; cephalic end bluntly rounded, abdomen gradually tapering posteriorly, caudal end rather pointed, armed with numerous elongate hooked spines, but without cremaster; maxillae more than half the length of wing cases; wing cases, antennae, and metathoracic legs nearly reaching sixth abdominal segment; antennae with tips separated, exposing ends of metathoracic legs.

RECURVARIA ROBINIELLA (FITCH)

Anacampsis robiniella Fitch, 1859, Rept. Nox. Benef. Ins. N. Y. 5: 834–835.
Recurvaria robiniella Busck, 1903, U. S. Natl. Mus. Proc. 25: 812, 813.

Twelve adults were reared March 17 to April 4, 1924, from larvae collected on *Amorpha fruticosa* Linnaeus at Lake Charlotte October 23, 1923. Feeding and pupation of the larva took place between two leaflets, which were webbed together along the edge so that the upper surfaces were on the inside. The species overwinters as pupae.

POLYHYMNO ACACIELLA BUSCK

Polyhymno acaciella Busck, 1900, Jour. N. Y. Ent. Soc. 8: 235–236, pl. 9, fig. 1; Busck, 1903, U. S. Natl. Mus. Proc. 25: 839; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 6179.

One adult was reared October 2, 1923, from leaves of *Vachellia farnesiana* Wight and Arnot collected in September, 1923, at Lake Charlotte.

GELECHIA MONOTAENIELLA, NEW SPECIES

(Fig. 2, A, B, C)

Gelechia monotaeniella, n. sp.

Antenna purplish black, with indistinct white annulations. Labial palpus with second joint whitish, dusted with black scales exteriorly; brush rather short, furrowed, gradually diminishing toward apex of second joint; terminal joint black, with few scattered white scales. Face, head, and thorax heavily overlaid with purplish-black scales. Fore wing dark fuscous, with few scattered black and brown scales; at apical fourth a transverse, irregular, incomplete, whitish fascia, before which are an ill-defined black costal spot and a similar dorsal spot; cilia light fuscous, with edging of black scales at apex. Hind wing silvery fuscous, dark toward apex; cilia light fuscous. Abdomen with basal segments above velvety yellowish, others above fuscous; under surface very dark fuscous. Legs dark fuscous, with tips of tarsi white.

Male genitalia of types figured (fig. 2, A, B, C).

Alar expanse.—12 to 16 mm.

Type.—Cat. No. 27337, United States National Museum.

Type locality.—North shore of Lake Charlotte, Chambers County, Tex.

Food plant.—*Vachellia farnesiana* (Linnaeus) Wight and Arnot.

Described from male type (Nov. 12, 1923) and 2 male and 16 female paratypes reared October 13 to November 12, 1923, and January 29 to March 24, 1924, from larvae collected from September to November, 1923.

This species is very near *serotinella* Busck, from which it is distinguished by the conspicuous whitish fascia on the fore wing, by lacking the narrow, chocolate brown, longitudinal streak below the costal edge of the fore wing (which is prominent in *serotinella*), and by its smaller size.

The larva is a leaf feeder, constructing a white silken tube along the midrib of a leaf or along a small branch and fastening to it leaflets of the host. The full-grown larva is 13 to 15 mm. long. Body pale

greenish white; upper surface with six broad, very irregular, wine-colored stripes, two dorsal, a subdorsal, and a lateral, the subdorsals being slightly widest; ventral surface with three similar but much pa er stripes, a narrow ventral and a broad, poorly defined marking on each side just above prolegs. Head pale yellow, posterior half reddish brown, with irregular, elongate, triangular markings of the same color projecting anteriorly; posterior margin narrowly black. Thoracic shield pale yellow, usually indistinctly bordered with black on posterior margin. Thoracic legs and anal plate yellow; abdominal legs normal, with alternating long and short crochets arranged in a complete circle; anal fork absent.

Pupation took place within thin, white, oval cocoons, to the outside of which were fastened particles of frass and leaflets of the host. A few of the specimens passed the winter as pupae, but most of them did not pupate until the first part of February. The colored stripes of the wintering larvae are barely visible.

The pupa is smooth, rather stout, 5 to 6 mm. long, and 1¾ to 2 mm. wide at the metathorax or widest part; cephalic end bluntly rounded; abdomen gradually tapering to caudal end, which is also bluntly rounded; maxillae very broad at base, two-thirds as long as wing cases; antennae meeting beyond maxillae but abruptly converging before end of wing cases, exposing ends of metathoracic legs; antennae, metathoracic legs, and wing cases reaching nearly to sixth abdominal segment; ventral posterior margin of seventh abdominal segment scalloped, and fringed with very short hairs; caudal end without cremaster.

A dipteron, *Zenillia blanda* Osten Sacken, emerged March 22, 1924, from a pupa of *Gelechia monotaeniella*. The parasite had pupated within the pupa of its host. A second parasitized pupa was noted, but the dipteron was not reared.

Two specimens of the hymenopteron *Brachymeria hammari* (Crawford) emerged October 8 and October 9, 1923, from two pupae of *Gelechia monotaeniella*. They were undoubtedly secondary parasites of the gelechiid, the primary host in this case being *Zenillia blanda*.

FAMILY BLASTOBASIDAE

ZENODOCHIUM CITRICOLELLA (CHAMBERS)

Blastobasis citricolella Chambers, 1880, U. S. Com. Agr. Rept. 1879: 206–207.
Zenodochium citricolella Dietz, 1910, Amer. Ent. Soc. Trans. 36: 11–12.

Several larvae and pupae of this species were taken in mature capsules of jimson weed (*Datura tatula* Linnaeus) at Smith Point, August 11, 1922. The larvae were webbing together and feeding upon the seeds. The pupae were in thin but strong webs, to the outside of which were fastened particles of frass and seeds. From this material five adults were obtained, the emergence dates ranging from August 17 to September 11, 1922.

HOLCOCERA CONFAMULELLA HEINRICH

Holcocera confamulella Heinrich, 1921, Jour. Agr. Research 20: 818–819.

Eight adults were reared during April, 1923, from fruits of *Crataegus* sp. collected at Smith Point October 18 and November 1, 1922.

FAMILY OLETHREUTIDAE

SUBFAMILY LASPEYRESIINAE

LASPEYRESIA PACKARDI (ZELLER)

Grapholitha packardi Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 300.
Steganoptycha pyricolana Murtfeldt, 1891, U. S. Dept. Agr. Bul. 23: 52.
Epinotia pyricolana Fernald, *in* Dyar, 1902, List N. Amer. Lepidop., no. 5234.
Enarmonia packardi Fernald, *in* Dyar, 1902, List N. Amer. Lepidop., no. 5282.
Enarmonia pyricolana Garman, 1918, Md. Agr. Expt. Sta. Bul. 223: 105–106, 108–109.
Laspeyresia packardi Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 7238.

A single male specimen was obtained April, 1923, from fruits of Crataegus collected at Smith Point during October of the preceding year. The above synonomy was furnished by Carl Heinrich.

SUBFAMILY EUCOSMINAE

EUCOSMA GIGANTEANA MINORATA HEINRICH

Eucosma giganteana minorata Heinrich, 1924, Jour. Wash. Acad. Sci. 14: 388.

In each of two flower heads of *Silphium gracile* A. Gray was found a dirty pinkish larva which might easily be mistaken for the pink bollworm. The part of the flower head above the cavity eaten out by the larva had darkened in color, indicating the presence of the insect. The specimens were collected near Liberty, July 17, 1922. Pupation of the one specimen reared took place July 19 in the cavity made during the growth of the larva and within a thin cocoon covered with small pieces of the dead portion of the flower head. The adult emerged July 29, 1922.

A second adult emerged July 5, 1923, from a pupa taken in a typically injured flower head of *Silphium gracile* at Stowell, Chambers County, July 3 of the same year. From material collected in mature and immature flower heads of the same plant at Liberty, July 7, 1923, a third moth was reared nine days later.

These adults furnished the type material for the variety *minorata* Heinrich.

EPIBLEMA TRIPARTITANA (ZELLER)

Paedisca tripartitana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 308.
Eucosma tripartitana Fernald, *in* Dyar, 1902, List N. Amer. Lepidop., no. 5141; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 7025.
Epiblema tripartitana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 146.

This is the olethreutid referred to in Heinrich's paper.[9] The host plant of this species is *Rudbeckia maxima* Nuttall, commonly called "niggerhead" in eastern Texas. The larvae are quite common in parts of Liberty, Chambers, and Harris Counties. The larva spends the first part of its life in a burrow in the central part of the flower head of the host plant, the cavity extending downward about an inch into the stem. The presence of the larva may often be detected by a slight enlargement of the stem immediately below the flower head.

[9] HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 813, illus. 1921.

The taking of a single specimen at light (Liberty, July 23, 1924) and the presence of a very few pupal cases in the flower heads of the hosts during July indicate a possibility of there being two generations. If such is the case, the following notes pertain to the second generation.

When apparently full grown the larva leaves its host through a small hole near the bottom of the cavity in the upper part of the stem and enters the same plant about a half inch above one of the lower leaves, from 6 to 12 inches above the surface of the soil. The leaf is webbed to the stem at the point of entrance of the larva. The conspicuous white chewings which have fallen to the ground at the base of the plant indicate that the larva has recently entered the stem. Upon entering the lower part of the plant the larva constructs a burrow which extends from a point 2 or 3 inches above the entrance hole to the base, where the cavity is slightly enlarged. The entrance hole is then plugged securely.

A few of the larvae had made the change described above before June 23, and by July 15 about half of them had reentered the plant at the base. Larvae were found in the flower heads of the host as late as October 10.

This insect passes the winter in the larval stage at the base of the host plant. At some time during the winter the larva cuts the stem off from the inside at the upper part of its cavity and plugs the upper inch or so with chewings. Just before pupation a hole is chewed nearly through the stem in the uppermost part of the remaining cavity for the emergence of the moth. Pupation takes place in the burrow. A number of pupae were observed to move quite rapidly down the burrow by means of a spiral movement of the abdomen.

The first pupa was taken at Liberty February 23, 1924, and from it the adult emerged March 15, 1924. From plants collected at the above locality on February 23 and 27, 1924, adults emerged March 15 to April 29 of the same year.

The larvae were parasitized by two species of Diptera: *Siphophyto floridensis* Townsend, four specimens of which were reared June 28 to July 3, 1923, and *Tachinophyto* sp., two specimens of which were reared June 29 and July 3, 1923.

The larvae were also parasitized by *Macrocentrus* sp. (Hymenoptera), the brownish cocoons of which were taken in large numbers in the flower heads of the host plant during June and July. Adults were reared June 29 to July 10. From a parasitized larva in the lower part of the stem of the host one specimen of *Bassus* sp. was reared March 29, 1924. The hymenopteron had spun its cocoon within the burrow made by the host.

EPIBLEMA DISCRETIVANA (HEINRICH)

Eucosma discretivana Heinrich, 1921, Jour. Agr. Research 20: 823–824.
Epiblema discretivana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 138, 147–148.

Larvae of this species were taken in stem galls of "wild myrtle" in August, 1924, with the assistance of A. C. Johnson, who collected and reared the type material of the species. The larvae were collected at Sheldon, Harris County, the type locality. The host plant has been determined as *Baccharis halimifolia* Linnaeus, of the family Carduaceae.

CROCIDOSEMA PLEBEIANA ZELLER

Crocidosema plebeiana Zeller, 1847, Isis von Oken 10: 721–722; Heinrich, 1921, Jour. Agr. Research 20: 822–823.

Larvae of this species were collected in several Malvaceae in addition to those mentioned by Heinrich.[10] From capsules of *Sida* sp. collected at Liberty July 31, 1922, several adults emerged during the first part of the following month. At Lake Charlotte on November 6 larvae were found in large numbers in the seed pods of *Sida spinosa* Linnaeus, *S. rhombifolia* Linnaeus, *Malvastrum americanum* (Linnaeus) Torrey, and *Anoda cristata* (Linnaeus) Schecht. Adults were reared during November, 1923, from larvae collected in mature capsules of *Reidlea corchorifolia* (Linnaeus) De Candolle at Smith Point November 1, 1923. This host plant belongs to the Buettneriaceae, and closely resembles some of the malvaceous plants.

The larvae were found singly in the seed capsules, except in the case of okra, in which several specimens were taken in one pod. In Sida and other host plants having small seed pods the larvae were often taken in a tie of the terminal leaves and immature capsules. The larvae pupated where feeding had taken place or within a roll or fold at the edge of a leaf. There are evidently two or more generations a year. Emergence dates ranged from the middle of August to the first part of December.

EPINOTIA PERPLEXANA (FERNALD)

Epiblema perplexana Fernald, 1901, Jour. N. Y. Ent. Soc. 9: 51.
Eucosma perplexana Fernald, *in* Dyar, 1902, List N. Amer. Lepidop., no. 5130; Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 6983.
Epinotia perplexana Heinrich, 1923, U. S. Natl. Mus. Bul. 123: 202–203.

Six adults of this species emerged October 16 to October 30, 1923, from mature pods of a wild cowpea, *Vigna repens* (Linnaeus) Kuntze, collected on the north shore of Galveston Bay at Smith Point October 3, 1923. The larvae fed upon the seeds and pupated within the pods.

SUBFAMILY OLETHREUTINAE

OLETHREUTES MALACHITANA (ZELLER)

Grapholitha malachitana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 292–293.
Olethreutes malachitana Dyar, 1902, List N. Amer. Lepidop., no. 5044.

A large series of moths was reared during October, 1922, from larvae collected in folded leaves of young persimmon trees (*Diospyros virginiana* Linnaeus), at Lake Charlotte September 22, 1922. One specimen of a parasitic dipteron, *Lixophaga variabilis* Coquillett, was reared September 26 from this material.

[10] HEINRICH, C. SOME LEPIDOPTERA LIKELY TO BE CONFUSED WITH THE PINK BOLLWORM. Jour. Agr. Research 20: 822, illus. 1921.

FAMILY TORTRICIDAE

PLATYNOTA FLAVEDANA CLEMENS

Platynota flavedana Clemens, 1860, Acad. Nat. Sci. Phila. Proc. [12]: 348; Dyar 1902, List N. Amer. Lepidop., no. 5382.

Three adults were obtained during the first season. One moth emerged August 12 from a pupa collected August 7 in loosely tied leaves of an undetermined plant at Liberty. Larvae were found webbing together and feeding upon the yellow ray flowers of *Helianthus* sp. at Smith Point September 14. One of these pupated in the loosely webbed ray flowers September 17, and the adult emerged September 26. A larva of *P. flavedana* was taken with larvae of *P. rostrana* (Walker) and *Sparganothis sulfureana* (Clemens) on *Eupatorium compositifolium* Walter at Smith Point September 14. This specimen pupated September 19, and the adult emerged September 26.

PLATYNOTA LABIOSANA ZELLER

Platynota labiosana Zeller, 1875, Verh. Zool.-Bot. Ges. Wien 25: 237–238; Dyar, 1902, List N. Amer. Lepidop., no. 5385.
Sparganothis labiosana Barnes and McDunnough, 1917, Check List Lepidop. Bor. Amer., no. 7326.

Six adults of this species were reared October 15 to November 5, 1923, from larvae collected in ties in the leaves of *Eupatorium compositifolium* Walter and of okra (*Hibiscus esculentus* Linnaeus) at Smith Point October 3, 1923.

PLATYNOTA ROSTRANA (WALKER)

Teras rostrana Walker, 1863, List Lepidop. Brit. Mus. 28: 290.
Platynota rostrana Dyar, 1902, List N. Amer. Lepidop., no. 5383; Heinrich, 1921, Jour. Agr. Research 20: 821.

The following records were made during 1922: One larva of *Platynota rostrana* was taken in a mature boll of *Hibiscus* sp. at Smith Point August 10, and on August 11 another specimen was taken in a mature seed capsule of *Datura tatula* Linnaeus at the same locality. The latter specimen was associated with the larvae of *Zenedochium citricolella* (Chambers). A full-grown larva was found in a seed of a mature boll of cotton which had been injured by the boll weevil, at Liberty September 8 (G. C. Albrecht, collector). Two moths were reared from larvae taken on *Eupatorium compositifolium* Walter at Smith Point September 14. The work of the larvae was very similar to that of a larva of *Sparganothis sulfureana* (Clemens) which was taken on the same plant. One larva was found feeding upon an injured orange at Lake Charlotte September 20 (E. Sherman, collector). Another specimen was taken in a withered flower of *Malvaviscus drummondii* Torrey and Gray at Smith Point October 19. Three larvae were found on leaves of *Hibiscus lasiocarpus* Cavanilles, two at Liberty September 12, and the third at Lake Charlotte October 27. One larva from each locality was in a rolled leaf the stem of which had been partially eaten through, apparently by the larva of *Gelechia hibiscella* Busck. Pupation dates of the above larvae ranged from August 11 to October 24, and emergence dates of the adults from August 19 to November 5, 1922.

Four adults were obtained during the second season. One moth was reared October 4 from a larva taken in a tie in the terminal leaves of *Amorpha fruticosa* Linnaeus at Lake Charlotte September 24. At the same locality and on the same date several larvae were collected in webbed leaflets of huisache, *Vachellia farnesiana* (Linnaeus) Wight and Arnot. From this material two adults were reared October 29 and November 6. The fourth adult emerged October 15 from a larva taken October 2 in a mature capsule of *Ipomoea speciosa* Walter at Smith Point.

FAMILY PHALONIIDAE

PHALONIA CEPHALANTHANA HEINRICH

Phalonia cephalanthana Heinrich, 1921, Jour. Agr. Research 20: 825–826.

Two adults were reared October 9 and October 11, 1922, from mature flower heads of *Cephalanthus occidentalis* Linnaeus collected in September, 1922, at Lake Charlotte. From material collected one year later at the same locality two moths emerged September 28 and September 29, 1923. Although the adults are from the type locality of the species, they are a lighter brown than the typical specimens.

Larvae of this species were found in the seed heads of the host plant at Uvalde during February, 1923, and at San Angelo in December of the same year, showing a general distribution over the State.

From the Lake Charlotte material the following Hymenoptera were reared: Three specimens of *Eupelmus momphae* Gahan (no date), and one specimen each of *Macrocentrus* sp. (Sept. 29, 1923) and *Apanteles* sp. (Oct. 23, 1923).

WATER AND WATER SOLUTIONS OF ORGANIC COMPOUNDS AS DIPS FOR THE SOIL OF POTTED PLANTS INFESTED WITH THE JAPANESE BEETLE [1]

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INTRODUCTION

The soil about the roots of plants grown in the open within the area infested by the Japanese beetle, *Popillia japonica* Newm., sometimes contains larvae of this insect (*8*, *9*, *17*, *18*, *20*),[2] yet large numbers of plants are grown within the infested area for commercial purposes under outdoor conditions. A Federal quarantine prohibits the shipment of such plants to points outside the infested zone unless the absence of the insect in the products in question is assured. Investigations were therefore undertaken at the Japanese Beetle Laboratory to develop methods for destroying the insect in the soil, with the object of applying these methods, wherever possible, to the treatment of the various types of plants grown commercially within the restricted area (*6*, *7*, *10*, *11*, *12*, *13*, *14*). Certain of the methods developed have been used successfully in the treatment of potted plants; for instance, the infestation of some varieties of plants growing in small pots has been prevented by mixing lead arsenate with the soil, and established infestations have been destroyed in tubs and the larger pots of ferns and other plants by percolating a dilute carbon disulphide emulsion through the soil. The treatment of pots smaller than 4 inches in diameter by the percolation method has not, however, been successful on account of the mechanical difficulties encountered and the cost of individual treatments of small pots in large quantities. The fumigation method (*13*), used so successfully with larger plants, could not be employed with smaller ones because of the mechanical difficulties. The experiments reported in this paper were made to determine whether it was economically possible to destroy an established infestation in the soil of several thousand small potted plants by dipping the pots in water or in toxic solutions.

WATER AS A DIP FOR SOIL INFESTED WITH LARVAE

It had been observed in the course of other related work that larvae were apparently uninjured by submersion in water for a period of 30 hours, but the maximum length of time that a larva could live under water was not known. Since water is so readily available for dipping purposes, it was deemed advisable to determine the period of submersion necessary to asphyxiate the larvae.

[1] Received for publication June 7, 1926; issued November, 1926. Contribution No. 9 of the Japanese Beetle Laboratory, Bureau of Entomology, Riverton, N. J., in cooperation with the Agricultural Departments of New Jersey, Pennsylvania, and Delaware.

[2] Reference is made by number (italic) to "Literature cited," p. 827.

The period of submersion lethal to the larvae was determined by placing 100 larvae, without soil, in individual wire cages submerged in water and removing 5 larvae on each subsequent day for examination. It was found that the larvae resisted asphyxiation by water for 15 days.

FACTORS INFLUENCING THE RESISTANCE OF LARVAE TO ASPHYXIATION

In the course of this experiment, it was observed that the larvae remained inactive when submerged in water and were surrounded to some extent by a film of air. It was thought probable that this trapped air assisted in prolonging the life of the larva, since it was possible that the air in the film was maintained at a sufficient oxygen tension to continue the larval metabolism. The experiment with water was therefore continued to determine whether the concentration of dissolved oxygen in the water affected the rate of mortality of the submerged larvae.

The influence of the concentration of the dissolved oxygen in the water was studied by sealing larvae in bottles of water of known oxygen content and at various intervals determining the larval mortality and the quantity of dissolved oxygen. One series of fifty 130 c. c. bottles was completely filled with aerated sterile distilled water containing 0.9 mgm. of dissolved oxygen per 50 c. c., and a second series of an equal number of bottles was filled with boiled sterile distilled water having 0.6 mgm. of dissolved oxygen. Five larvae were submerged in the water of each bottle. The bottles were then sealed by means of rubber stoppers and paraffin and kept at a temperature of 70° F. On certain subsequent days two bottles of water containing initially 0.9 mgm. of dissolved oxygen and two bottles containing initially 0.6 mgm. were opened to determine the effect on the larvae [3] and the oxygen content of the water.[4]

The mortality of the larvae, as shown in Table 1, was practically the same in the water containing 0.9 mgm. of dissolved oxygen as in that containing 0.6 mgm. Submersion for 15 days was necessary to kill the most resistant individuals in both concentrations of oxygen. The results indicate that there is no correlation between the initial quantity of dissolved oxygen in the water and the rate of mortality of the submerged larvae.

[3] The larvae removed for observation were placed on the surface of moist soil. If a larva became active and burrowed into the soil within 48 hours subsequent to its removal from the water, it was considered as unaffected by the submersion; if it showed signs of life but failed to go normally into the soil, it was regarded as injured by the treatment: and if it exhibited no evidence of life, it was held under observation until the first indication of tissue disintegration before it was pronounced dead.

[4] The method used for determining the dissolved oxygen in the water was a modification of that recommended by the Association of Official Agricultural Chemists for the determination of dissolved oxygen in potable water (*1*, *p.* 89). The procedure is briefly as follows: Before removing the larvae from a bottle, two 50 c. c. samples of water were withdrawn by means of a 50 c. c. pipette and discharged into 250 c. c. Erlenmeyer flasks, the delivery end of the pipette being placed on the bottom of the flasks to preclude the incorporation of atmospheric oxygen in the flowing water. Two c. c. of manganous sulphate solution and 2 c. c. of sodium hydroxide-potassium iodide solution were added below the surface of the water by means of pipettes and mixed by gentle whirling to avoid undue agitation of the surface of the water. Sodium hydroxide reacts with manganous sulphate to form manganous hydroxide ($Mn(OH)_2$) which, having a great affinity for oxygen, unites with the dissolved oxygen in the water to form manganous acid (H_2MnO_3). This acid in contact with alkaline manganous hydroxide is converted into a brown flocculent precipitate of the manganous manganites ($Mn.MnO_3$ or $Mn.H_2(MnO_3)2$. Stoppers were placed in the flasks until the precipitate of the manganites had settled. Two c. c. of 50 per cent sulphuric acid was then added. The hydriodic acid, formed by the reaction of sulphuric acid with potassium iodide, decomposed the manganite precipitate with the liberation of free iodine in direct proportion to the quantity of dissolved oxygen originally present in the water. The contents of the flask were then titrated against N/40 sodium thiosulphate, 1 c. c. of which was equivalent to 0.2 mgm. of dissolved oxygen at 0° C. and 760 mm. pressure, according to the usual iodometric procedure.

TABLE 1.—*Relation of dissolved oxygen in water to larval mortality*

Number of days larvae were submerged	Using boiled distilled water				Using aerated distilled water			
	Dissolved oxygen per 50 c. c. (average)	Larvae dead	Larvae injured	Larvae unaffected	Dissolved oxygen per 50 c. c. (average)	Larvae dead	Larvae injured	Larvae unaffected
	Mgm.				*Mgm.*			
0	0.55	0	0	5	0.89	0	0	5
	.60	0	0	5	.92	0	0	5
7	.53	0	0	5	.44	0	0	5
	.40	1	0	4	.41	0	0	5
9	.38	2	0	3	.47	1	0	4
	.44	1	0	4	.51	0	0	5
12	.38	1	0	4	.48	1	0	4
	.39	1	0	4	.47	1	0	4
13	.20	3	0	2	.34	1	0	4
	.27	1	0	4	.29	0	0	5
14	.16	5	0	0	.17	4	1	0
	.18	4	1	0	.20	5	0	0
15	.17	5	0	0	.20	5	0	0
	.20	5	0	0	.11	5	0	0
16	.17	5	0	0	.20	5	0	0
	.17	5	0	0	.20	5	0	0

This resistance of Popillia larvae to asphyxiation is not unusual among insects. It has been recognized for a number of years that insects are difficult to kill by suffocation. As early as 1670, Boyle (*3, p. 2051–2054*) confined grasshoppers, caterpillars, beetles, and flies in the best vacuum which he could obtain and found that the insects recovered when returned to air. Treviranus (*19, p. 151*) observed great variation among insects in their oxygen requirements. Bourcart (*2, p. 30*) states that it was necessary to submerge a field infested with soil insects for a relatively long period, 8 to 60 days, in order to asphyxiate them. Shafer (*16*) more recently found that insects could survive submersion in atmospheres of carbon dioxide and hydrogen for several hours.

When the results of the experiments with the Popillia larvae are considered from the standpoint of their practical application, it is seen that the necessary period of submersion is too prolonged to be employed. Submersion in water is therefore of little apparent value as a means of destroying the larvae in the soil of small potted plants. In view of these results, a series of experiments was begun to determine the value of water solutions of organic compounds as dips for the small potted plants infested with larvae.

COMPARATIVE VALUE OF WATER SOLUTIONS OF ORGANIC COMPOUNDS AS DIPS FOR INFESTED SOIL

Previous experiments (*5, 7, 10, 12, 14*) have shown that the larvae of the Japanese beetle are killed when removed from soil and submerged in water solutions or emulsions of such compounds as sodium cyanide, thymol, carbon disulphide, o-cresol, phenol, benzyl chloride, nitrobenzene, naphthalene, and others. Certain of these toxic solutions, prepared according to a procedure previously employed by the writer (*7*) were tested to determine their relative effectiveness in killing submerged larvae embedded in small masses of soil.

The results obtained by the method of experimentation necessarily chosen in classifying the few selected compounds are not conclusive,

but only indicate the compound worthy of more exhaustive tests. Ten liters of each dipping solution and 10 liters of tap water were prepared and held in suitable containers at a temperature of approximately 70° F. Eight of the previously infested 2-inch flowerpots of soil were submerged in each solution.[5] At time intervals of 6, 12, 24, and 30 hours two pots were taken from each solution to observe its effect and that of the period of submersion on the larvae. The larvae were removed from the treated soil as soon as it was withdrawn from the dipping solutions and were placed in pots of untreated soil for subsequent examination.

The results of these dipping tests, which were obtained 72 hours after the last pot was removed from the solutions, are summarized in Table 2. Carbon disulphide emulsion was the most effective of the compounds penetrating in lethal concentration into the infested soil.

TABLE 2.—*Ability of dilute organic emulsions to penetrate into submerged pots of soil and kill the larvae contained therein*

Test No.	Toxic agent of the emulsion	Concentration of toxic agent	Effect on larvae submerged at 70° F.							
			6 hours		12 hours		24 hours		30 hours	
			Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
		Gm. per liter								
1-8	o-Cresol ($CH_3C_6H_4OH$)	2.0	2	0	2	0	1	1	2	0
			2	0	1	1	1	1	0	2
9-16	do	1.0	2	0	1	1	1	1	2	0
			2	0	2	0	1	1	0	2
17-24	Phenol (C_6H_5OH)	2.0	2	0	2	0	1	1	1	1
			1	1	1	1	1	1	1	1
25-32	do	1.0	1	1	2	0	1	1	1	1
			1	1	1	1	1	1	1	1
33-40	Benzyl chloride ($C_6H_5CH_2Cl$)	3.5	2	0	2	0	2	0	2	0
			2	0	2	0	1	1	2	0
41-48	do	2.0	1	1	1	1	1	1	1	1
			1	1	2	0	1	1	2	0
49-56	Carbon disulphide (CS_2)	2.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
57-64	do	1.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
65-72	Nitrobenzene ($C_6H_5NO_2$)	3.5	2	0	2	0	2	0	0	2
			2	0	2	0	2	0	2	0
73-80	Sodium cyanide (NaCN)	2.0	2	0	2	0	2	0	2	0
			2	0	2	0	2	0	2	0
81-88	do	1.0	2	0	2	0	1	1	2	0
			2	0	2	0	2	0	1	1
89-96	Water only		0	2	1	1	2	0	0	2
			1	1	1	1	0	2	0	2
97-99	Control (soil not submerged)								0	2
									0	2
									0	2

CARBON DISULPHIDE EMULSION AS A DIP FOR SOIL INFESTED WITH LARVAE

The previous experiments indicated that dilute carbon disulphide emulsion might be effective as a dip in destroying the infestation in such small masses of soil as are contained in 2-inch and in 4-inch

[5] Three weeks prior to the actual treatment, 70 gm. of rich, moist greenhouse soil was placed in each of 100 2-inch flowerpots and infested with two third-instar larvae. The pots were maintained at the customary greenhouse temperature, 50° to 70° F., and sprinkled daily with water. As a result, the soil was compact and the larvae in a normal active state when the pots were submerged in the toxic solutions.

flowerpots. It was determined therefore to test further the efficiency of this emulsion by treating a large number of salvia and nasturtium plants growing in 2-inch pots and pine and arbor vitae growing in 4-inch pots.

EFFICIENCY OF EMULSION WITH 2-INCH POTS

Emulsions were prepared for each series of plants in progressive dosages, namely, 0.125, 0.250, 0.375, 0.500, 0.625, 0.750, 0.875, 1.000, 1.125, 1.250, and 1.375 c. c. of carbon disulphide per liter of solution. Ten liters of each concentration of the emulsion and 10 liters of untreated water, serving as a control, were placed in suitable containers and held at a temperature of approximately 70° F. throughout the submersion period.

Ten uniform plants of each series of salvia were selected, weighed, and submerged in each concentration of the carbon disulphide and in the water control so that the surface of the soil was just below the surface of the liquid. In the case of the nasturtiums, a certain proportion of which had failed to grow, it was necessary to reduce the number of plants in each solution from 10 to 5. When the submersion had been prolonged for 18 hours, the pots were removed from the solutions, freed of excess water, and reweighed. They were then returned to the greenhouse for subsequent observation to determine the indirect effect of the various concentrations on the aerial portions of the plants.

After a period of 4 to 6 weeks the aerial portions of the plants usually showed signs of stimulation, or injury, or were dead. The ball of soil about the roots of each plant was then carefully removed from the pot and crumbled to find the larvae. If a larva had been killed, it was often difficult to locate even a fragment of its body; but if it was unaffected or had been only slightly injured, it was usually very active and easily found.

The results of these tests, recorded in Table 3, indicate that there is practically no margin between the dosage of carbon disulphide in the dipping solution lethal to the larva and that injurious to the plant. Complete larval mortality was secured in series 1 with a dosage of 1.0 c. c. of carbon disulphide per liter; only 0.75 c. c. was required in series 2, whereas 1.375 c. c. was not sufficient in series 3. A dosage of 1.25 c. c., however, was fatal to salvia and nasturtium. This wide variation in the concentration necessary to kill the larvae is probably caused by the difference in the individual activity and location of the insects in the mass of soil. Larvae which are active or which are in the layers of soil in close proximity to the sides of the flowerpot are more readily killed than those which have burrowed into the center of the soil mass.

EFFICIENCY OF EMULSION WITH 4-INCH POTS

Although the results with the 2-inch potted plants were negative, it was deemed advisable to repeat the experiments with 4-inch pots. Pine and arbor vitae, growing in 4-inch pots of infested soil, were therefore subjected to the same treatment as that received by the plants in the 2-inch pots. The results of these tests were also negative.

TABLE 3.—*Carbon disulphide emulsion as an insecticidal dip for soil of plants in 2-inch pots submerged for 18 hours at 70° F.*

Series No.	Pot No.	Concentration of CS_2	Solution imbibed by soil			Effect on larvae			Species of plant	Effect on plants
			Minimum in 10 pots	Maximum in 10 pots	Average in 10 pots	Number dead in 10 pots	Number alive in 10 pots	Per cent dead in 10 pots		
		C. c. per liter	*Gm.*	*Gm.*	*Per cent*					
	1–10	0.125	0.2	10.4	2.58	0	20	0	Salvia	Stimulated.
	11–20	.250	1.9	8.3	2.48	0	20	0	do	Do.
	21–30	.375	2.1	13.6	3.28	0	20	0	do	Do.
	31–40	.500	1.1	7.7	2.40	4	16	20	do	Do.
	41–50	.625	1.0	11.6	3.30	6	14	30	do	Unaffected.
	51–60	.750	4.5	11.2	4.37	8	12	40	do	Sickly.
	61–70	.875	1.5	8.7	2.45	19	1	95	do	40 per cent dead.
1	71–80	1.000	1.4	15.3	3.51	20	0	100	do	70 per cent dead.
	81–90	1.125	1.2	10.0	2.53	20	0	100	do	80 per cent dead.
	91–100	1.250	2.8	13.2	3.57	20	0	100	do	100 per cent dead.
	101–110	1.375	2.5	10.0	3.92	20	0	100	do	Do.
	111–120	[1]	.7	16.3	3.76	1	19	5	do	Unaffected.
	121–125	.125	2.8	10.6	4.19	0	10	0	Nasturtium	Do.
	126–130	.250	8.1	37.3	11.40	3	7	30	do	Do.
	131–135	.375	11.7	35.4	7.70	1	9	10	do	Do.
	136–140	.500	11.1	15.8	7.52	8	2	80	do	Do.
	141–145	.625	11.5	27.2	9.42	8	2	80	do	Do.
	146–150	.750	19.1	20.9	12.72	10	0	100	do	Do.
	151–155	.875	14.2	31.1	10.55	10	0	100	do	Sickly.
2	156–160	1.000	15.0	25.2	8.23	10	0	100	do	20 per cent dead.
	161–165	1.125	10.3	21.0	7.73	10	0	100	do	80 per cent dead.
	166–170	1.250	12.2	29.9	9.13	10	0	100	do	100 per cent dead.
	171–175	1.375	11.0	21.0	9.04	10	0	100	do	Do.
	176–180	[1]	11.0	16.0	6.43	0	10	0	do	Unaffected.
	181–190	.125	8.7	14.5	6.42	0	20	0	Salvia	Stimulated.
	191–200	.250	10.0	29.4	10.66	0	20	0	do	Do.
	201–210	.375	10.0	25.0	8.65	5	15	25	do	Do.
	211–220	.500	11.0	28.0	10.00	9	11	45	do	Unaffected.
	221–230	.625	13.0	25.0	10.28	4	16	20	do	20 per cent sickly.
	231–240	.750	40.0	19.0	6.52	6	14	30	do	60 per cent sickly.
3	241–250	.875	5.0	22.0	8.71	8	12	40	do	Do.
	251–260	1.000	5.0	29.0	9.36	11	9	55	do	90 per cent dead.
	261–270	1.125	18.0	26.0	13.11	19	1	95	do	100 per cent dead.
	271–280	1.250	12.0	25.0	9.65	14	6	70	do	Do.
	281–290	1.375	5.0	20.0	6.04	12	8	60	do	Do.
	291–300	[1]	7.0	26.0	9.34	0	20	0	do	Unaffected.

[1] None (control).

DISCUSSION OF SOIL CONDITIONS LIMITING THE EFFECTIVENESS OF CARBON DISULPHIDE EMULSION IN SUBMERGED SOIL

The larvicidal action of a dilute carbon-disulphide emulsion under these conditions is dependent upon the volume of the solution penetrating into the soil and upon the length of time during which the active principle of the solution maintains its potency in contact with the soil. The pore space is occupied by water and air. If the water content of the soil is low the air space is large. Under optimum conditions for growing plants, over half of the pore space is filled with water. It is apparent therefore that the entire volume of solution imbibed by the soil from even an unlimited source would necessarily be limited.

The toxic solution which penetrates into the soil is markedly affected by the soil particles. It is well known that dissolved as well as suspended material can be more or less completely removed from water by passing the solution through sand or soil. This removal of the solute or suspended material from water is the result of absorption, the chemical reaction between the soil and the dissolved substance, and of adsorption, the condensation or concentration of the dissolved substance on or about the surface of the soil particles (*4, p. 59–69; 15, p. 349–374*). The dissolved or suspended substance is not uniformly distributed throughout the soil by the water and tends to become concentrated in the outer layers of the soil mass. Even in the concentrated area as well as in other parts of the soil mass a certain portion of the toxic solute is rendered impotent by being absorbed in the moisture film surrounding the soil particles. The diffusion of the toxic agent into the soil to replace that portion of the imbibed solution removed by adsorption is a slow process and is of little value in killing larvae. The results obtained by Leach and Thomson (*10*) with infested soil dipped in toxic solutions gave every indication that the portion of the initially imbibed solution which was unaffected by absorption or adsorption was responsible for the insect mortality. The effective action of the carbon-disulphide emulsion is dependent therefore upon the initial concentration obtained throughout the soil mass.

It is apparent from the brief discussion of the soil conditions which influence the insecticidal efficiency of the carbon-disulphide emulsion in the submerged soil, that the action of a toxic dipping solution is uncertain and almost entirely dependent upon favorable soil conditions. The extreme variability in these conditions would make rather dubious the general use of any dipping solution.

SUMMARY

Experiments were made to determine whether an established infestation of the Japanese beetle in the soil of small potted plants could be destroyed by submersion for a definite period in water or in water solutions (emulsions) of o-cresol, phenol, benzyl chloride, carbon disulphide, nitrobenzene, and sodium cyanide.

The larvae resisted asphyxiation in water for 15 days. No correlation was found between the rate of larval mortality and the concentration of the dissolved oxygen of the water.

Carbon-disulphide emulsion was the most effective of the solutions in killing the larvae in the submerged soil. The insecticidal concentrations, however, usually injured plants.

It was apparent in these experiments that the effective action of any dipping solution is so dependent upon soil imbibition, soil adsorption, and soil absorption as to limit the application of even an effective insecticide to conditions where these factors are favorable.

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NUTRITIVE VALUE OF PROTEIN IN BEEF EXTRACT, OX BLOOD, OX PALATES, CALF LUNGS, HOG SNOUTS, AND CRACKLINGS [1]

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INTRODUCTION

The purpose of the investigation herein reported was to determine the nutritive value of the protein in beef extract, ox blood, ox palates, calf lungs, hog snouts, and cracklings, as measured by the growth induced in albino rats. In these experiments, as in previous studies with other meat products (*4*, *5*),[3] the term "protein" is used in a general sense to include all organic nitrogenous compounds, whether true proteins or not. In most meat products only a small proportion of the nitrogen is a constituent of nonproteins, but in beef extract at least one-half of the nitrogen is in that form.

DESCRIPTION OF PRODUCTS

In the United States beef extract is an important product of the meat-canning industry. It is prepared by concentrating in vacuum kettles the clear broth obtained from cooking fresh beef. Two types of extract are prepared, depending on the degree to which the beef broth has been concentrated, viz, fluid extract, which contains approximately 50 per cent moisture, and solid extract, which contains approximately 25 per cent moisture.

In addition to beef extract, similar extracts are prepared from various edible parts and organs of cattle, sheep, and hogs, and from the broth obtained from cooking corned beef preparatory to canning. These extracts must be labeled to show their true character, and the term "beef extract" is restricted to that product which has been prepared entirely from fresh beef.

Blood is an important potential source of food for man and animals. As collected in the packing house it is equal to approximately 10 per cent of the dressed weight of fat cattle, 8 per cent of the weight of sheep, and 4 per cent of the weight of hogs. On account of a natural distaste for blood as a food, however, it is used to a very limited extent in the human diet, chiefly as an ingredient of sausage, but an appreciable quantity of the dried product is fed to farm animals. Only blood from cattle is used as human food.

Ox palates, commonly called "hard palates," comprise the tissues in the roofs of the mouths of cattle. This product consists largely of rather tough connective tissue, together with some muscle and fatty tissue. Ox palates are first cooked and then utilized in the manufacture of sausage. They are used only to a limited extent for human food.

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[2] The writers extend their thanks to Warren L. Briggs, junior scientific aid of the Biochemic Division, for assistance in the care of the experimental animals used in these investigations and for the preparation of the graphs.

[3] Reference is made by number (italic) to "Literature cited," p. 842.

Calf lungs, as well as those of cattle and sheep, find limited use in the human dietary.

Cracklings are the residue from the rendering of lard or beef fat in open kettles. They consist largely of connective tissue, together with a small proportion of muscle and variable quantities of fat. This product is used to a limited extent as human food, the bulk of it being utilized either for feeding farm animals or as a constituent of fertilizers.

Hog snouts, which comprise the facial tissues on the upper jaw extending from the eyes to the tip of the nose, consist almost entirely of connective tissue and fat. This product is used chiefly in the preparation of potted products, sausage, and similar articles

PREVIOUS INVESTIGATIONS

BEEF EXTRACT

Beef extract has been the subject of numerous investigations, but most of these have related to its physiological effects, and relatively little work has been done to determine the nutritive value of the nitrogenous compounds in this product. Although approximately one-half of the nitrogen in beef extract is present as a constituent of meat bases which have no food value, the remainder is found chiefly in the form of proteoses, peptones, amino acids, and gelatin; compounds which might be expected to have considerable food value.

Thompson (*10*) fed dog biscuit to dogs until they remained constant in weight. Beef extract was then added to the diet. The dogs increased in weight after the addition of the beef extract, but returned to their former weights when the extract was omitted. A part of the nitrogen in the beef extract was retained by the dogs, but the most of it was excreted in the urine. The beef extract induced a more complete digestion of the dog biscuit, as evidenced by a reduction in the quantity and the nitrogen content of the feces.

Thompson, Caldwell, and Wallace (*11*), in experiments with human subjects and with dogs, found that the addition of beef extract to the diet caused an increase in weight and the retention by the body of a considerable proportion of the nitrogen in the extract. There was also a decreased excretion of nitrogen in the feces, due apparently to the better digestion of the ordinary diet. These writers concluded, therefore, that beef extract has both a direct and an indirect nutritive value.

Hutchison (*6, p. 94, 95*) states that "rats which were fed on 4 grammes of meat extract daily died quite as soon as other rats which got no food at all." He states that "[beef] extract, though containing too little protein matter to be in itself a food, may act as a valuable adjuvant to other foods, especially where appetite and digestion are feeble."

BLOOD

A considerable number of feeding experiments with blood have been conducted with farm animals at the agricultural experiment stations in this country and in Canada, but it does not seem desirable to discuss these in detail. It will suffice to quote briefly from a recent text by Henry and Morrison (*3, p. 186, 309, 375, 652*) regarding the value of blood as a food for farm animals.

Dried blood is particularly useful with young pigs and calves, as a skim milk substitute, or for sickly animals. Its usual high price stands in the way of its common use for other animals, but it has been fed with success to horses, dairy cows, and sheep.

Tankage and blood meal were found by Burkett at the North Carolina station to be useful for run-down, thin horses, 1 to 2 lbs. of tankage or 1 lb. of blood meal being employed. Pott states that blood meal has given excellent results in horse feeding. As such animal by-products are unpalatable to horses, they must be mixed with well-liked feeds.

Blood meal is not commonly used for feeding dairy cows, primarily due to its high price, but it is quite extensively used in calf feeding. It is occasionally used in stimulating cows to their utmost when on test, but only 1 to 2 lbs. should be added to the ration. It is not palatable to cattle, though they can be taught to eat it when mixed with other feeds. In a trial by Lindsey at the Massachusetts station 1.1 lbs. blood meal was equal to 2.2 lbs. cottonseed meal.

Blood meal is used but relatively little for swine feeding, except sometimes for very young pigs, as tankage is usually a more economical source of protein.

Imabuchi (*7*) determined the digestibility of dried blood in a test with a dog. The animal was first fed a ration consisting of 300 gm. of horse meat and 50 gm. of fat meat for a period of 5 days; then a ration composed of 46 gm. of dried blood, 100 gm. of horse meat, and 50 gm. of fat meat for a period of 10 days, and finally the first-named ration for a period of 5 days. The digestibility of the protein in the rations fed during the three periods was as follows: Period 1, 94.3 per cent; period 2, 86.6 per cent; and period 3, 94 per cent. The dog was in nitrogen equilibrium during the first period but lost 3.3 gm. of nitrogen, corresponding to 117 gm. of flesh, during the second period when blood was included in the ration.

Blum (*1*) states that blood serum is utilized practically as completely as meat, but that the red corpuscles have a much lower value, the protein in the latter being only 70 to 75 per cent digestible. Whole blood is considered to have a somewhat lower nutritive value than meat.

Opel (*9, p. 141–147*) states that the protein in ox blood was found by Salkowski to be 95 per cent digestible and that the protein in sheep blood was 96.5 per cent digestible, as determined by Beck, the experiments being conducted with dogs. Opel states also that serum protein is digested as thoroughly as that in meat, but that the protein in the corpuscles is not so completely digested, 25 to 30 per cent of the nitrogen appearing in the feces.

Hutchinson (*6, p. 72, 73*) states that two French experimenters found that 2 pounds daily of blood was not sufficient to maintain life in dogs for more than a month. Hemoglobin, which makes up the larger part of the protein in blood, is reported to be not well utilized.

OX PALATES

The digestibility of ox palates was determined by Langworthy and Holmes (*8*). The palates were first cooked and then fed to men as the chief source of protein in a mixed diet. The digestibility of the total protein in the diet was found to be 87.3 per cent, and that in the ox palates alone was calculated as being approximately 86.8 per cent. It is stated that the protein of cooked hard palates is somewhat less thoroughly digested than that of the common cuts of meat.

OTHER PRODUCTS

Although no experimental evidence concerning the nutritive value of the protein in calf lungs, hog snouts, or cracklings was found in the

literature, the following statements indicate the apparent nutritive value of these products:

Hutchison (*6, p. 73*) states that lungs "are sometimes eaten, but can not be regarded as a really good form of food." They are described as being largely composed of an elastic material, an albuminoid, and as being imperfectly digested and useless as building material in the body.

Pork cracklings, according to Henry and Morrison (*3, p. 186*) are fully as valuable as tankage for swine. Bull (*2, p. 219*) states that cracklings are considered more digestible than tankage and that the two products have about the same value as feed for hogs. He also recommends cracklings as a feed for poultry.

EXPERIMENTAL PROCEDURE

METHODS

The methods employed in this investigation are essentially the same as those followed by the writers in previous studies with other meat products (*4, 5*). Each meat product under examination constituted the only source of nitrogen in a ration which also contained adequate quantities of vitamins A, B, and D and of mineral matter. As a rule, each ration was fed to six young, male, albino rats weighing approximately 40 gm. each at the start, but in a few instances mature rats of both sexes were used. Except where indicated on the graphs, the curves denote the growth made by male rats.

DESCRIPTION OF SAMPLES

The beef extract, which was of the solid or paste variety, was obtained direct from a factory. The extract was dissolved in water, mixed with starch, and the mixture was dried and ground so as to facilitate incorporation in the ration.

Defibrinated ox blood was dried in the laboratory in a current of air at a temperature under 70° C. All the dried product was found to be practically soluble in water. A portion of the dried blood was heated 30 minutes in an autoclave under 15 pounds' steam pressure.

The dried serum and hemoglobin were obtained from a factory which prepares these products on a commercial scale. The serum was prepared so as to eliminate fibrin.

The ox palates were first cooked, then ground, dried in a current of air at 60° to 70° C., and again ground; the calf lungs were prepared in a similar manner except that they were not cooked.

The hog snouts were ground, heated on a steam bath to remove most of the fat, and the tissue residue was dried in a current of air and then ground fine.

Two lots of pork cracklings, each of which contained only a little muscle tissue, were obtained from different packing houses. The cracklings were ground fine before use in the rations.

COMPOSITION OF MEAT PRODUCTS

The proximate composition of the samples of meat products is reported in Table 1. The percentage of crude protein was obtained by multiplying the percentage of nitrogen by 6.25. These results do not necessarily indicate the true protein content of each product, certainly not of the beef extract, but they offer a convenient basis for

comparing the nutritive values of the mixed nitrogenous compounds in the several products.

TABLE 1.—*Composition of dried meat products* [a]

Laboratory No.	Product	Moisture	Ash	Nitrogen	Crude protein (nitrogen ×6. 25)	Fat
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1256	Beef extract [b]	14. 32	21. 75	11. 27	70. 44	
1186	Ox blood			14. 40	90. 00	0. 79
1232	Ox blood (heated in autoclave)			14. 59	91. 19	
1214	Serum	10. 01	13. 57	10. 75	67. 19	.34
1237	Serum		13. 35	11. 59	72. 43	
1213	Hemoglobin	15. 12	1. 35	13. 28	83. 00	.34
1255	Ox palates			10. 05	62. 81	32. 99
1287	Calf lungs			12. 11	75. 69	6. 93
1258	Hog snouts			14. 01	87. 56	7. 65
1269	Cracklings			13. 86	86. 62	3. 48
1284	Cracklings			14. 35	89. 69	6. 79

[a] The chief object of these analyses was to determine the nitrogen and fat; in some cases the moisture and ash were determined also.
[b] The beef extract was not dried.

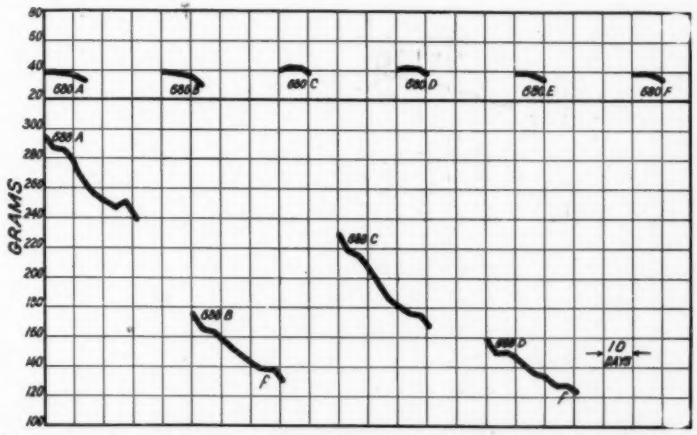

FIG. 1.—Growth curves of rats fed a ration containing 18 per cent of crude protein (nitrogen×6.25) from beef extract

RESULTS OF FEEDING EXPERIMENTS

BEEF EXTRACT

A ration containing 18 per cent of crude protein (nitrogen×6.25) from beef extract, but which probably contained less than 9 per cent of true protein, was fed to 6 young male rats and to 4 mature ones. The results of these tests are reported in Figure 1 and Table 2, the two groups of rats being numbered 680 and 688, respectively. From Figure 1 it is evident that the ration was very inadequate, even for maintenance, for either group of rats. It was possible to continue the experiment with the mature rats for a period of 31 days, but each rat lost weight steadily throughout the test, the losses ranging from 19.5 to 27.4 per cent. Food consumption was approximately normal for the rats in each group.

In this connection it may be stated that a ration containing 5 per cent of protein of good quality and 10 per cent of fat is adequate for maintenance in rats, whereas 10 per cent of good protein will induce approximately normal growth.

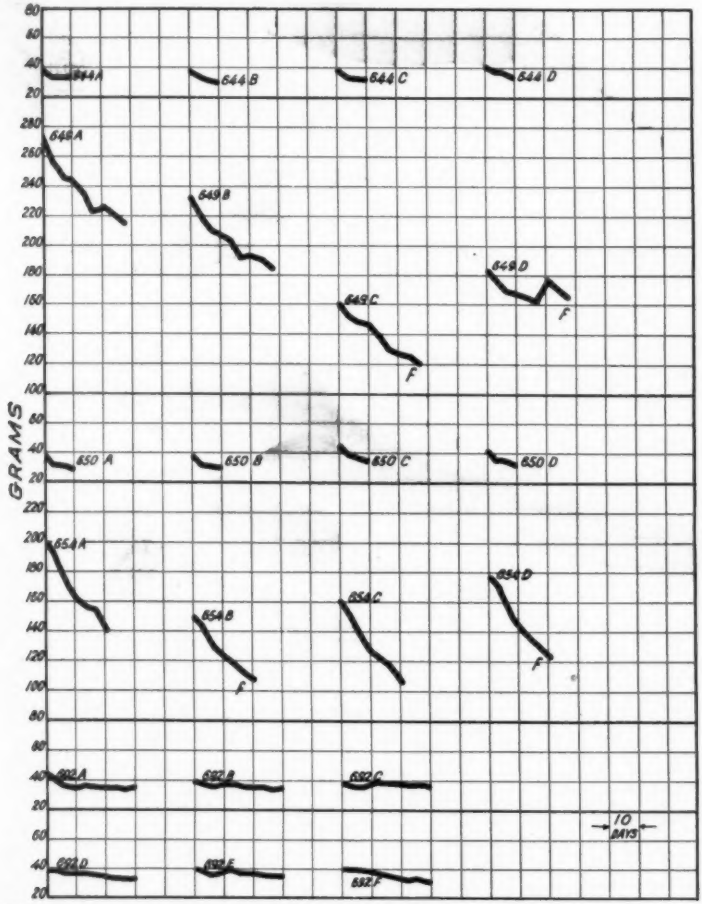

FIG. 2.—Growth curves of rats fed rations containing dried ox blood as the source of protein. Rats numbered 644 and 649, respectively, received 20 per cent protein; rats numbered 650 and 654, respectively, 50 per cent protein; and rats numbered 692, 15 per cent protein

OX BLOOD

The results of the feeding tests with ox blood are reported in Figure 2 and in Table 2. Four young male rats, numbered 644, were fed a ration containing 20 per cent of crude protein from dried, defibrinated blood which had been heated in an autoclave after drying. It is apparent that the ration was inadequate even for maintenance (fig. 2), but on referring to Table 2 it will be noted that, with one exception, the food consumption of the rats was very

low. The average daily food intake for each of three rats ranged from 1.7 to 1.9 gm.—quantities far below their normal requirements—but the average daily food intake of the fourth rat, 3.9 gm. was practically normal.

The same ration was fed to four mature rats numbered 649 (fig. 2 and Table 2), and it will be noted that they lost weight more rapidly than the young rats. Although the ration contained adequate quantities of vitamins A, B, and D, each rat was fed daily four drops of cod-liver oil. From Table 2 it may be noted that the food consumption of these rats also was below normal.

A ration containing 50 per cent of protein from dried raw blood was fed to young male rats numbered 650 (fig. 2 and Table 2). Each rat received 4 drops of cod-liver oil daily in addition to 2 per cent of the oil already present in the ration. All of these rats lost weight throughout the test. The same ration was fed to 4 mature rats numbered 654 (fig. 2 and Table 2), but the loss in weight was more pronounced than in the young rats. The average daily food intake for the rats in each group was much below the maintenance requirement.

A ration containing 15 per cent of protein from dried raw blood was fed 6 young male rats numbered 692, and each rat was fed daily 0.1 gm. vitamin B from yeast and 5 drops of cod-liver oil in addition to the quantities of these vitamins already present in the ration. From Figure 2 it is evident that these rats nearly maintained their weight; but in Table 2 it will be noted that the food intake was low

TABLE 2.—*Results of feeding tests with beef extract, ox blood, serum, and hemoglobin as sources of protein in the diet of rats*

Source of protein	Protein in ration	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (−) in weight	Total feed consumed	Gain in weight per gram of protein consumed	
								30 days	60 days
	Per cent		*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Beef extract	18	680 A	15	38	32	−6	53		
Do	18	680 B	15	39	30	−9	47		
Do	18	680 C	11	40	38	−2	44		
Do	18	680 D	11	41	37	−4	41		
Do	18	680 E	11	39	34	−5	36		
Do	18	680 F	11	39	34	−5	35		
Do	18	688 A	31	297	239	−58	330		
Do	18	688 B	31	176	130	−46	183		
Do	18	688 C	31	230	167	−63	186		
Do	18	688 D	31	160	123	−37	153		
Ox blood (heated in autoclave)	20	644 A	10	38	34	−4	18		
Do	20	644 B	10	38	30	−10	17		
Do	20	644 C	10	39	33	−6	19		
Do	20	644 D	10	41	33	−8	39		
Do	20	649 A	28	274	215	−59	144		
Do	20	649 B	28	234	185	−49	139		
Do	20	649 C	28	161	121	−40	81		
Do	20	649 D	28	184	164	−20	139		
Ox blood	50	650 A	10	38	29	−9	16		
Do	50	650 B	10	38	30	−8	16		
Do	50	650 C	10	45	34	−9	19		
Do	50	650 D	10	42	32	−10	26		
Do	50	654 A	21	202	139	−63	61		
Do	50	654 B	21	150	107	−43	61		
Do	50	654 C	21	161	105	−56	35		
Do	50	654 D	21	178	122	−56	53		
Do	15	692 A	30	44	35	−9	45		
Do	15	962 B	30	39	34	−5	54		
Do	15	692 C	30	38	36	−2	56		
Do	15	692 D	30	39	33	−6	48		
Do	15	962 E	30	40	35	−5	48		
Do	15	692 F	30	40	31	−9	67		

TABLE 2.—*Results of feeding tests with beef extract, ox blood, serum, and hemoglobin as sources of protein in the diet of rats*—Continued

Source of protein	Protein in ration	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (−) in weight	Total feed consumed	Gain in weight per gram of protein consumed	
								30 days	60 days
	Per cent		*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Serum No. 1214	10	623 A	32	38	70	+32	153	2.11	
Do	10	623 B	42	38	58	+20	134	1.62	
Do	10	623 C	32	42	97	+55	214	2.71	
Do	10	623 D	32	40	65	+25	149	1.80	
Do	10	623 E	32	40	65	+25	133	2.02	
Do	10	623 F	32	38	72	+34	154	2.18	
Average gains						+32		2.07	
Serum No. 1237	10	656 A	30	43	46	+3	109		
Do	10	656 B	30	40	39	−1	94		
Do	10	656 C	28	40	45	+5	88		
Do	10	656 D	21	45	49	+4	78		
Serum No. 1214	15	637 A	62	45	100	+55	324	1.94	1.21
Do	15	637 B	62	38	140	+102	405	2.53	1.70
Do	15	637 C	62	41	173	+132	471	2.39	1.90
Do	15	637 D	62	40	122	+82	383	2.48	1.47
Do	15	637 E	62	40	138	+98	413	2.29	1.62
Do	15	637 F	62	39	166	+127	441	2.33	1.94
Average gains						+99		2.33	1.64
Hemoglobin	10	624 A	14	38	32	−6	35		
Do	10	624 B	14	39	30	−9	38		
Do	10	624 C	14	40	32	−8	36		
Do	10	624 D	13	40	32	−8	31		

OX SERUM

Six young rats numbered 623 (fig 3 and Table 2) were fed a ration containing 10 per cent of serum protein. The rats grew at a rate far below normal for rats getting a ration containing 10 per cent of protein of good quality, such as that in veal (fig. 4). The rats fed the serum protein made an average gain in weight of only 2.07 gm. for each gram of protein consumed, as compared with a gain of 2.86 gm. for the rats getting 10 per cent of veal protein (Table 3).

A ration containing 10 per cent of protein from another lot of serum from the same factory was fed to rats numbered 656 (fig. 3 and Table 2), but this ration was scarcely more than adequate for maintenance.

Rats numbered 637 (fig. 3 and Table 2) were fed a ration containing 15 per cent of protein from the same lot of serum as that fed to rats No. 623. This ration induced fair but not normal growth. These rats made an average gain in weight of 2.33 gm. for each gram of protein consumed during a 30-day period, as compared with a gain of 2.86 gm. for rats fed 10 per cent of veal protein during a like period (Table 3).

OX HEMOGLOBIN

A ration containing 10 per cent of hemoglobin was fed to rats numbered 624 (fig. 3 and Table 2). This ration proved to be inadequate even for maintenance, although food consumption was not much below normal.

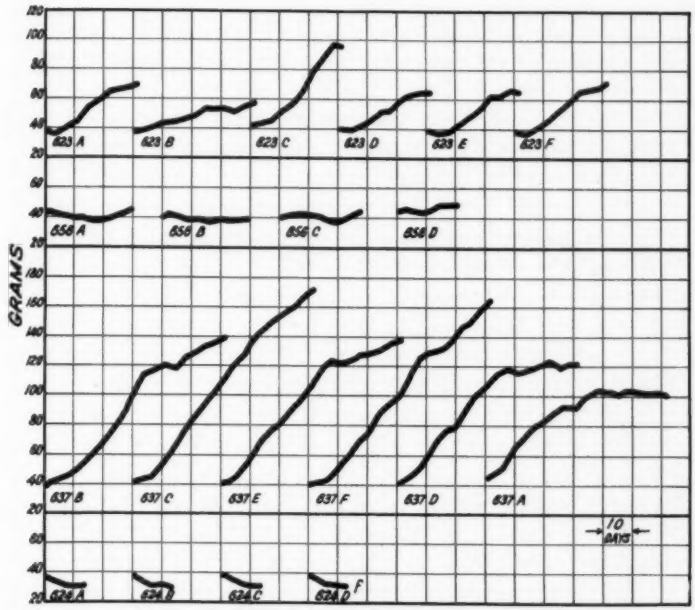

FIG. 3.—Growth curves of rats fed dried ox serum and hemoglobin as the sources of protein in the rations. Rats numbered 623 and 656, respectively, were fed 10 per cent of protein from two different lots of serums. Rats numbered 637 received 15 per cent of serum protein, and rats numbered 624 were fed 10 per cent hemoglobin

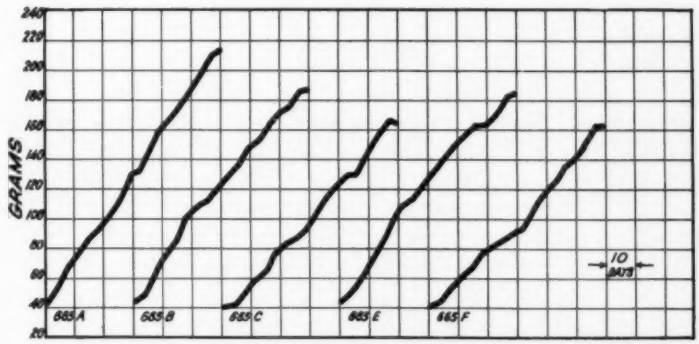

FIG. 4.—Growth curves of rats fed a ration containing 10 per cent of protein from veal

OX PALATES

Rations containing 10 and 15 per cent, respectively, of protein from ox palates were fed to rats numbered 678 and 679 (fig. 5 and Table 3). The rats getting 10 per cent of protein grew very slowly and those receiving 15 per cent made only slightly better growth. During the

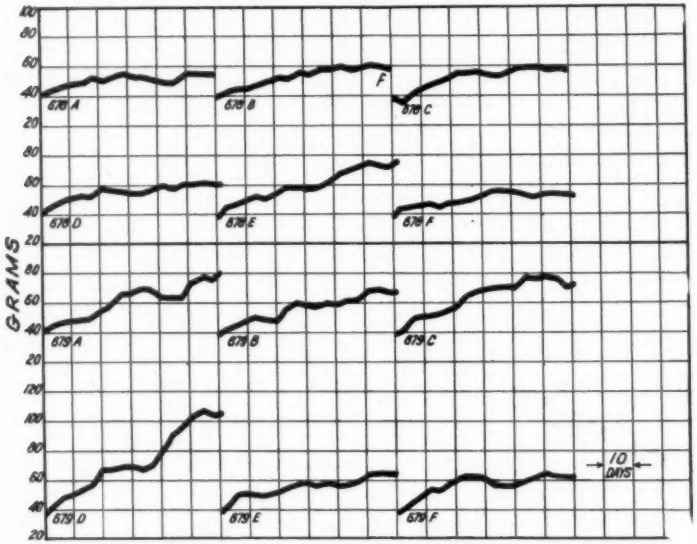

FIG. 5.—Growth curves of rats fed ox palates as the source of protein in their diet. Rats numbered 678 received 10 per cent, and rats numbered 679, 15 per cent protein

first 30 days of the test the rats receiving 10 per cent protein gained an average of only 1.22 gm. in weight for each gram of protein consumed, and those getting 15 per cent protein only 1.14 gm., as compared with a gain of 2.86 gm. for rats getting 10 per cent veal protein during a like period.

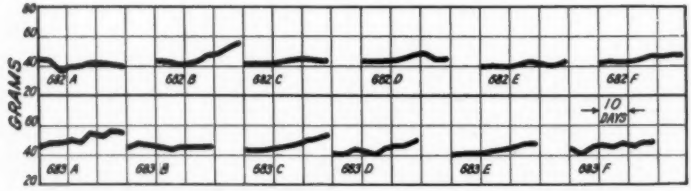

FIG. 6.—Growth curves of rats fed hog snouts as the source of protein in their diet. Rats numbered 682 received 10 per cent, and rats numbered 683, 15 per cent protein

HOG SNOUTS

Rats numbered 682 and 683, respectively, were fed rations containing 10 and 15 per cent of protein from hog snouts (fig. 6 and Table 3). The first group of rats made practically no growth, and the second group made only slight growth during a period of 29 days. Food consumption was approximately normal.

PORK CRACKLINGS

Two lots of cracklings were fed to rats numbered 697 and 731, respectively, the first group receiving 15 per cent protein, the second group 20 per cent (fig. 7 and Table 3). It is evident that these rations were only approximately adequate for maintenance, the first group making a slight average gain and the second group a slight average

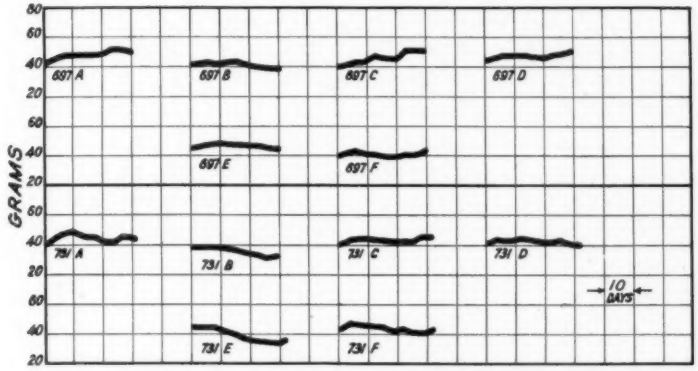

FIG. 7.—Growth curves of rats fed pork cracklings as the source of protein in their diet. Rats numbered 697 were fed a ration containing 15 per cent of protein from one lot of cracklings; rats numbered 731 received 20 per cent of protein from another lot of cracklings

loss. It may be recalled that 5 per cent of protein of good quality is adequate for maintenance and 10 per cent for approximately normal growth.

CALF LUNGS

A ration containing 10 per cent of protein from calf lungs was fed to rats numbered 725 (fig. 8 and Table 3). These rats made fair,

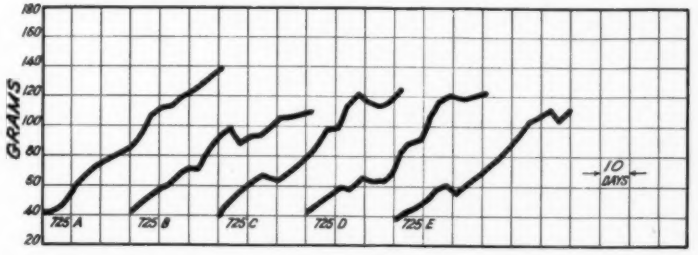

FIG. 8.—Growth curves of rats fed a ration containing 10 per cent of protein from calf lungs

though somewhat subnormal, growth. The growth curves are rather irregular, but the reason is not apparent. From Table 3 it appears that the rats getting 10 per cent of protein from calf lungs made an average gain in weight of 2.02 gm. for each gram of protein consumed during a period of 30 days as compared with a gain of 2.86 gm. for the rats getting 10 per cent of veal protein.

TABLE 3.—*Results of feeding tests with ox palates, hog snouts, cracklings, and calf lungs as sources of protein in the diet of rats*

Source of protein	Protein in ration	Rat No.	Duration of test	Initial weight	Final weight	Gain (+) or loss (−) in weight	Total feed consumed	Gain in weight per gram of protein consumed	
								30 days	60 days
	Per cent		*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Ox palates	10	678 A	60	40	55	+15	229	1.03	0.66
Do	10	678 B	60	39	58	+19	205	1.28	.93
Do	10	678 C	60	39	57	+18	248	1.17	.73
Do	10	678 D	62	40	61	+21	263	1.11	.82
Do	10	678 E	62	38	76	+38	296	1.50	1.18
Do	10	678 F	61	38	52	+14	208	1.20	.73
Average gains						+21		1.22	.84
Ox palates	15	679 A	61	39	81	+42	309	1.23	.90
Do	15	679 B	61	38	67	+29	253	1.01	.77
Do	15	679 C	61	39	72	+33	302	1.19	.72
Do	15	679 D	61	38	106	+68	361	1.32	1.26
Do	15	679 E	60	39	64	+25	244	.97	.68
Do	15	679 F	60	38	63	+25	265	1.10	.63
Average gains						+37		1.14	.83
Hog snouts	10	682 A	29	45	40	−5	118		
Do	10	682 B	29	43	56	+13	127		
Do	10	682 C	29	41	44	+3	115		
Do	10	682 D	29	43	45	+2	112		
Do	10	682 E	29	39	43	+4	107		
Do	10	682 F	29	42	47	+5	121		
Do	15	683 A	29	45	55	+10	124		
Do	15	683 B	29	45	46	+1	109		
Do	15	683 C	29	43	54	+11	123		
Do	15	683 D	29	41	50	+9	123		
Do	15	683 E	29	40	47	+7	106		
Do	15	683 F	29	45	49	+4	128		
Cracklings No. 1269	15	697 A	30	43	50	+7	100		
Do	15	697 B	30	42	39	−3	80		
Do	15	697 C	30	40	51	+11	100		
Do	15	697 D	30	45	51	+6	92		
Do	15	697 E	30	45	45		91		
Do	15	697 F	30	40	43	+3	90		
Cracklings No. 1284	20	731 A	31	39	44	+5	106		
Do	20	731 B	29	39	33	−6	96		
Do	20	731 C	32	40	46	+6	98		
Do	20	731 D	32	41	40	−1	129		
Do	20	731 E	33	45	37	−8	109		
Do	20	731 F	32	43	43		115		
Calf lungs	10	725 A	61	40	140	+100	549	2.10	1.84
Do	10	725 B	62	42	110	+78	445	2.23	1.55
Do	10	725 C	62	39	125	+86	461	2.06	1.83
Do	10	725 D	62	42	122	+80	444	1.75	1.83
Do	10	725 E	60	38	112	+74	381	1.97	1.94
Average gains						+84		2.02	1.80
Veal	10	665 A	60	41	214	+173	715	3.05	2.42
Do	10	665 B	60	44	188	+144	629	2.90	2.29
Do	10	665 C	60	40	165	+125	522	2.90	2.39
Do	10	665 E	60	44	185	+141	629	2.92	2.24
Do	10	665 F	60	41	163	+122	505	2.53	2.42
Average gains						+141		2.86	2.35

DISCUSSION OF RESULTS

In the experiments reported in this paper each meat product was the only source of protein in a ration which was adequate in other respects for growth, and the results obtained indicate the apparent value of a product as a source of protein for maintenance and growth only under these conditions. Although a protein may be inadequate

for growth when used alone in a ration, owing to a deficiency of one or more essential amino acids, the same protein may give much better results when fed in combination with other proteins which supply the lacking amino acids.

BEEF EXTRACT

A ration containing 18 per cent of so-called "crude protein" (nitrogen×6.25) from beef extract, but in reality only approximately 9 per cent of true protein compounds, was fed to young and to mature rats with very unsatisfactory results. In neither instance was this ration adequate even for maintenance, much less for growth, although only 5 per cent of protein of good quality is required for maintenance.

Although it appears that the nitrogenous compounds in beef extract are of poor nutritive quality, it is not to be inferred that the product has no value in the diet. Beef extract represents in high concentration the flavoring constituents of beef, and for that reason it has a distinct value for culinary purposes. Beef extract is also rich in phosphates, but its chief value appears to lie in the stimulating effect which it exerts upon the digestive processes.

BLOOD

The experiments with both dried blood and hemoglobin are unsatisfactory as a measure of the nutritive value of the protein in these products on account of the dislike which the rats showed for them. This aversion was indicated by the behavior of the rats but more particularly by the record of the food intake. Various expedients were used to increase food consumption, but without much success. It appears that the albino rat is not a satisfactory animal for the estimation of the nutritive value of dried blood and hemoglobin, and that the information must be obtained with other animals.

The experiments with dried serum show that 10 per cent of protein from that source was sufficient only for very slow growth in rats, but 15 per cent protein induced fairly satisfactory, though not normal growth. Since 10 per cent of muscle protein is practically sufficient for normal growth, it appears that serum protein has a much lower nutritive value.

OX PALATES

The experiments reported indicate that the protein in ox palates is deficient in certain essential amino acids, since rations containing 10 and 15 per cent [4] respectively, of protein from this source induced only very slow growth. These results were to have been expected since ox palates consist chiefly of connective tissue with a small proportion of muscle. The fact that the protein in ox palates is fairly well digested (*8*) is, of course, not a correct indication of its nutritive value.

HOG SNOUTS

Rations containing 10 and 15 per cent, respectively, of protein from hog snouts were only slightly more than adequate for maintenance purposes. Since the hog snouts used consisted almost entirely of connective tissue and fat, and apparently no muscle tissue, these results were to have been expected.

[4] Ten per cent of animal protein of good quality, such as that in muscle tissue, is practically adequate for normal growth in albino rats.

CRACKLINGS

Rations containing 15 and 20 per cent, respectively, of protein from two lots of cracklings proved to be only barely adequate for maintenance. Since these cracklings consisted chiefly of connective tissue and only a very little muscle tissue, the results are as expected. In view of these findings, statements that cracklings have as high nutritive value as tankage for feeding hogs seem open to question.

SUMMARY

In this paper are reported the results of feeding tests with rats to determine the relative nutritive values of the proteins in several meat products when each was the sole source of protein in the diet.

The total nitrogenous compounds in beef extract were found to have a very low value for maintenance and growth in rats. A ration containing 18 per cent of so-called "crude protein" (nitrogen × 6.25), or approximately 9 per cent of true protein, was inadequate for maintenance.

Dried blood and hemoglobin were very distasteful to rats, and food consumption was so low that no statement concerning the nutritive value of the protein in these products is warranted.

Serum protein had a considerably lower nutritive value than muscle protein. Fifteen per cent of serum protein was somewhat inadequate for optimal growth.

The protein in ox palates was evidently deficient in certain amino acids, since 15 per cent of protein from this source induced only very slow growth.

Hog snouts and pork cracklings also contained incomplete proteins, since 15 per cent of protein from either source was adequate only for maintenance.

The protein in calf lungs was of fair quality, 10 per cent being sufficient for moderate growth.

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THE GROWTH OF OPHIOBOLUS GRAMINIS SACC. IN RELATION TO HYDROGEN-ION CONCENTRATION [1]

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INTRODUCTION

Several investigators have observed an apparent influence of soil reaction on the occurrence and severity of the take-all disease of wheat caused by *Ophiobolus graminis* Sacc. In general, it has been considered that alkaline soils favor the disease, but this point has not been definitely settled. A study of the literature shows that the experimental data dealing with this problem are rather incomplete, and that the vague term "alkaline soil" generally refers to soil to which alkaline substances, particularly lime, have been added at one time or another. With the exception of one case, all observations have been made in the field and, as no hydrogen-ion determinations have been made for the soils in question, it is impossible to correlate the occurrence and severity of the disease with active acidity or alkalinity.

Recently, Kirby (*7*, *8*) [3] and Davis (*4*) have studied the influence of the reaction of nutritive media on the growth of the parasite in pure culture. Kirby concluded that the fungus grows best on a highly alkaline medium, whereas Davis's results indicate that a neutral or slightly acid medium is most favorable for growth. Their experiments were rather limited, and in view of this fact it is difficult to draw definite conclusions from their results. The work on this phase of the subject has been summarized by McKinney (*9*).

In order to gain additional information on this problem and to explain, if possible, some of the apparent inconsistencies, it seemed important first to conduct a series of experiments with the parasite in pure culture. Accordingly, the growth of the fungus has been studied under different hydrogen-ion concentrations on each of several synthetic and nonsynthetic types of liquid and solid media and under different environmental conditions. The results of these experiments are presented in this paper.

MATERIALS AND TECHNIQUE

ORGANISM

A single-spore strain of *Ophiobolus graminis* was used throughout this study. The fungus was isolated by R. S. Kirby from a single ascospore obtained from a wheat plant growing in the State of New York and affected with the take-all disease. A culture of this single-spore isolation was furnished to H. H. McKinney. He and R. J. Davis have kept the fungus in prime condition by repeated passage

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[3] Reference is made by number (italic) to "Literature cited," p. 871.

through wheat plants and by single ascopore isolations from the fungus growing in pure culture.

Potato-dextrose agar proved to be the most favorable medium, and stock cultures were made in both test tubes and Petri dishes. The medium was made in the usual way: Potatoes 300 gm., dextrose 30 gm., agar 20 gm., water 1,000 c. c. All stock cultures were allowed to grow at room temperature either in the dark or in very subdued light, and only young and vigorous growing cultures were used as sources of inoculum. An incubation interval of 14 to 21 days for stock cultures proved satisfactory for all purposes.

METHOD OF CULTURE

The stock nutrient media for the experiments reported in this paper were made on the basis of a "2X" concentration; that is, 500 c. c. of water were used instead of 1,000 c. c. as specified in the formulae. This method, which has been described in detail by Karrer and Webb (*6*), permits dilutions of the various solutions by the addition of regulated quantities of acid or alkali and water for the adjustment of hydrogen-ion concentrations without disturbing the concentrations of the various constituents. The acid and alkali employed were HCl and NaOH. A strength of N/5 was very satisfactory always for the acid and, in most cases, for the alkali. In a few instances, however, when relatively large quantities of alkali were desired, a strength of N/1 was necessary.

Erlenmeyer culture flasks of 150 c. c. capacity were used, and in all cases each flask contained 50 c. c. of the nutrient solution. A 25 c. c. portion of the particular (2X) culture medium was pipetted into each flask, and sufficient water to equal the difference between 50 c. c. and the sum of the volumes of the nutrient medium and the acid or alkali was added. The flasks were plugged with cotton and autoclaved for 30 minutes at 15 pounds pressure. Similarly, the acid and the alkali were sterilized separately. After cooling, the cultures were removed to a culture chamber and definite quantities of sterile acid and alkali were added with sterile, graduated pipettes. The separate sterilization of the acid, the alkali, and the nutrient medium minimizes any reaction on the part of the sugar. Moreover, the final volume of the culture solutions in each case represents a dilution of the constituents comparable with that specified in the original formula. The solutions were allowed to stand at least for a period of 24 hours in order to reach a state of equilibrium before the hydrogen-ion determinations were made. Certain color transformations occurred from the addition of alkali, but these will be discussed later.

Similar methods were adopted for the solid media. Large quantities of the 2X culture fluid to which 2 per cent bacto agar had been added were pipetted, while warm, into 1,000 c. c. flasks. Calculated quantities of water were added to each, the flasks were plugged, and the cultures were sterilized intermittently on three successive days for a period of 15 minutes at 13 pounds pressure. After the third sterilization and before cooling, regulated quantities of acid or alkali were added to the flasks and the solutions were vigorously shaken. These stock solutions then were poured into sterile Petri dishes, and usually there were 6 to 8 plates for each reaction. The plates employed were 9 cm. in diameter and the volume of medium in each

ranged from 20 to 25 c. c. Hydrogen-ion determinations were made immediately with the remaining portions of the stock solutions. Transformations in color and difficulties with setting were encountered in certain instances, but these will be discussed later. Usually 6 to 8 liters of the 2X solution or of the 2X agar were made at the same time, so all the data represent uniformity for a particular medium.

All operations pertaining to the culture work were performed in a culture chamber, and every precaution was taken to prevent contaminations. The interior parts of the culture chamber were washed with 1:1,000 $HgCl_2$ and the cultures and all necessary apparatus were then deposited. Dust particles within the chamber were precipitated by means of live steam flowing into the chamber for a period of several minutes. During long operations, live steam was permitted to flow periodically and no cultures were disturbed until everything had become settled. It is of interest to note that only about 2 per cent of the flask cultures became contaminated. Petri-dish cultures showed a somewhat higher percentage of contamination on account of the fact that the covers were removed daily to permit accurate measurements of the colonies.

CULTURE MEDIA

The adjusted P_H values for each of the liquid and solid media used are given in Table 1, together with the quantity of acid or alkali necessary for such adjustment.

The formula for the potato-dextrose decoction is as follows: 300 gm. of potatoes, 30 gm. of dextrose, and 1,000 c. c. of water. The potatoes were peeled, cut into small pieces, autoclaved for one hour at 15 pounds pressure, and filtered through absorbent cotton while hot. Dextrose was added, the solution was thoroughly stirred, and regulated portions were pipetted into flasks. The decoction always tested P_H 5.7 or P_H 5.8, and the buffer action was very good. With a dilution of 10 times, the decoction tested P_H 6.0, and with a dilution of 30 times, it tested P_H 6.2.

Czapek's full nutrient solution was made according to the formula used by Karrer and Webb (*6*): $MgSO_4$, 0.5 gm.; KH_2PO_4, 1.0 gm.; KCl, 0.5 gm.; $NaNO_3$, 2.0 gm.; $FeSO_4$, 0.01 gm.; cane sugar 30 gm.; H_2O, 1,000 c. c. This nutrient solution also was used in two modified forms. In one case nucleic acid was substituted for the sodium nitrate. In the other case, caramelization was produced by prolonged autoclaving as follows: A large quantity of the nutrient solution was made upon a basis of 10X concentration, autoclaved for two or four hours at 15 pounds pressure, diluted to 2X, and pipetted into the culture flasks.

The corn-meal decoction was prepared in the following manner: 150 gm. of corn meal were added to 1,000 c. c. of water. The mixture was then heated over a flame, brought to a slow boil, filtered twice through two thicknesses of cheesecloth, and finally filtered through absorbent cotton.

Solid media were made in all cases by the single addition of bacto agar to the liquid media at the rate of 20 gm. per liter.

A knowledge of the factors or conditions which stimulate growth in the alkaline cultures of potato-dextrose agar should prove valuable. Reference is made to the increasing browning with increasing additions

of alkali. This in itself indicated that chemical changes were taking place, but little definite information relative to the occurrence and the degree of such changes in culture media is available.

TABLE 1.—*Liquid and solid media adjusted to various hydrogen-ion concentrations by regulated additions of N/5 hydrochloric acid or N/5 sodium hydroxide*

2X solution	HCl or NaOH N/5	H_2O	Total	Hydrogen-ion concentration, P_H								
				Potato dextrose		Corn meal		Czapek's solution	Czapek's nucleic acid		Czapek's caramelized	
				Decoction	2 per cent agar	Decoction	2 per cent agar		Solution	2 per cent agar	Solution	2 per cent agar
	HCl											
C. c.	*C. c.*	*C. c.*	*C. c.*									
25	5.00	20.00	50	2.50	3.20					2.3		
25	4.00	21.00	50	2.95	3.40							2.3
25	3.50	21.50	50					2.0		2.5		
25	3.00	22.00	50	3.50	3.70							
25	2.50	22.50	50									2.6
25	2.00	23.00	50	4.00	4.10					3.0		
25	1.20	23.80	50									3.1
25	1.00	24.00	50					2.6	2.6	3.6	2.5	
25	.75	24.25	50	4.85	4.60	2.70						
25	.60	24.40	50			3.20						3.4
25	.50	24.50	50									
25	.40	24.60	50			3.50		3.0	3.6	4.7		
25	.30	24.70	50			3.60	4.0				3.0	3.7
25	.20	24.80	50			3.90	4.4	3.5	4.2	5.1		
25	.10	24.90	50			4.20	5.2				3.3	3.8
25	.00	25.00	50	5.80	5.30	5.30	5.8	4.6	5.5	5.5	3.3	3.9
	NaOH											
25	.05	24.95	50			6.60	6.8					
25	.10	24.90	50			6.80	7.1	5.5	5.7	5.7	3.4	4.3
25	.20	24.80	50			7.80	7.5				3.6	
25	.30	24.70	50	6.50	5.60	8.20	8.1					5.1
25	.40	24.60	50								4.7	
25	.50	24.50	50	6.95	5.80	8.50	8.3					
25	.60	24.40	50									5.7
25	.75	24.25	50	7.40	6.70	9.00	8.4	6.6	6.6	6.5	5.5	
25	1.00	24.00	50								5.8	6.3
25	1.10	23.90	50	7.70				7.0				
25	1.30	23.70	50						7.0	6.7		
25	1.50	23.50	50	8.30							6.5	6.6
25	1.60	23.40	50					7.6				
25	1.75	23.25	50		7.00				7.9	6.9		
25	1.90	23.10	50					8.0				
25	2.00	23.00	50	8.40	8.00	10.30	9.0		8.4	7.4	6.8	6.9
25	2.50	22.50	50	8.80				8.7				
25	3.00	22.00	50						9.5	8.5	7.3	7.8
25	4.00	21.00	50			11.20	9.3	9.8+			9.2	8.8
25	5.00	20.00	50	9.50	9.10			9.8+	10.8	9.5		
25	6.00	19.00	50			11.80	9.8	9.8+			9.6	9.5
25	7.00	18.00	50						11.4	10.1		
25	8.00	17.00	50	9.6+	9.50	12.10	10.1				10.2	
25	10.00	15.00	50	9.6++	9.60		10.4		11.7	10.5	10.7	9.6
25	15.00	10.00	50				10.8			11.3	11.0	9.7
25	16.65	8.35	50		9.60							
25	20.00	5.00	50				11.7					
25	20.80	4.20	50		9.60							
25	25.00	0.00	50		10.30					11.8		10.9

HYDROGEN-ION DETERMINATIONS

Hydrogen-ion determinations of all liquid media were made at room temperature and of all solid media at 60° to 70° C. by both the colorimetric and electrometric methods, as described in detail by Clark (*3*). The liquid media were allowed to stand 24 hours at room temperature before being tested. The various types of solid media were held in a water bath at 60° to 70° C., following the last steril-

ization and subsequent additions of either acid or alkali, and were tested as soon as possible. Such factors as time and temperature proved extremely important. The presence of color necessitated the use of a comparator and colorimeter, the intensity of the color determining which to use. A standard instrument, modified and calibrated to meet the requirements of this work in accordance with the method described by Duggar (*5*), was employed. Czapek's solution gave a bluish-green color upon the addition of the bromphenol blue indicator, and it was impossible to get good agreements between these colors and those of the buffer standard solutions, particularly near P_H 3.0. Electrometric determinations were made for the cultures beyond the alkaline range of the indicators, and sufficient electrometric tests also were made on the culture solutions and on the standard buffer solutions to verify the colorimetric method.

The methods employed for the final hydrogen-ion values of the substrate were similar to those for the initial determinations. The fungus generally produced a definite mat in the favorable cultures of the liquid media, and filtration therefore was simple. The filtrates were tested both colorimetrically and electrometrically. In the case of the solid media, the substrates were cut into small pieces and the agar blocks from duplicate cultures were transferred to clean Erlenmeyer flasks. The flasks were placed in a water bath with a temperature of 60° to 70° C. and kept there until the substrates were liquefied. The fragments of fungous mats floated to the surface and were removed.

Marked changes in the reaction of alkaline solid media occurred at the temperatures employed for liquefaction of the agar. The changes varied more or less directly with the amount of alkali present, the temperature employed, and the time involved. When agar cultures were kept in a water bath at 60 to 70° C. while the hydrogen-ion determinations were being made, it soon became apparent that the electrometric and colorimetric methods of determination gave different results. A period of 15 to 20 minutes generally was necessary for equilibrium to be reached by the electrometric method, and the values so obtained were usually a few tenths of a P_H less than those obtained immediately by the colorimetric method. If, however, colorimetric determinations were made at the time that the electrometric readings were made, the values of the two methods were practically identical. More uniform results were obtained when the cultures were kept in a water bath at a constant temperature of 60° C. than when they were subjected to live steam in an Arnold sterilizer for short periods. Intense browning occurred in the most alkaline cultures, and rather high dilutions were necessary for the colorimetric determinations. The immediate colorimetric figures are believed to represent more nearly the exact reactions of the poured plates than do the delayed electrometric values. The speed with which colorimetric determinations can be made therefore reduces the time factor to a minimum, and this is a decided point in favor of this method for agar determinations. It is regrettable that the range of indicators is limited on the alkaline side and that wider application of this method is thus prevented at the present time.

The hydrogen-ion values in the tables represent initial and final determinatins for both inoculations and controls. Reactions extending almost throughout the entire range were obtained with each

medium, and especial attention has been given to the study of the influence of slight acidity, of neutrality, and of slight alkalinity on the growth of the organism. Aside from these desired concentrations, the range has been conveniently but not equally subdivided.

INOCULATION AND INCUBATION

The inoculum consisted of young mycelium grown on potato-dextrose agar in Petri dishes. The age of the cultures generally varied from 14 to 21 days, and the mycelium was always taken from a concentric zone about 2 cm. in width near the periphery of the fungus colony. Agar containing such mycelium was cut into small blocks about 4 mm. square, and one such inoculum block was placed in each culture. With the liquid media, some of the blocks would naturally sink and others would not. However, by rotating the flasks vigorously, all of the blocks were made to sink. The growth data for the liquid media, therefore, represent growth which initially was submerged.

Similarly, an inoculum block was placed on the surface of the agar in the center of each Petri dish. Adequate uninoculated controls were included for the experiments involving both liquid and solid media.

The cultures for the most part were incubated in large glass chambers located in a greenhouse, and the temperature and humidity in these chambers were controlled by automatic regulators. The cultures were placed in single rows on shelves near one of the glass walls of the chamber and were protected from direct sunlight by shades and screens. Certain of the cultures were incubated in the dark. Heavy black paper was properly cut, fitted around each flask, and held in place by a rubber band at the neck of the flask. Black hoods made of the same material were placed over these flasks and all light was thus excluded. The hoods were removed each day for a few minutes in order to promote aeration more or less comparable with that in the unhooded cultures. The Petri-dish cultures were placed in sterile paper bags and incubated in the laboratory incubators; the bags were removed under sterile culture-chamber conditions for a few minutes each day while growth data for the organism were being recorded.

During the late spring, temperatures were not easily controlled in the greenhouse chambers, and it became necessary to employ exclusively the incubators located in the laboratories. The temperatures in these incubators were regulated within 1 degree, but there was no control for humidity, and the cultures grew in total darkness.

CHEMICALS

High-grade chemicals were used throughout this investigation, and the most careful technic was employed in all instances. To eliminate the high acidity of the monobasic potassium phosphate, this salt was recrystalized until the Sörensen coefficient of P_H 4.529 for 1/15 molecular solution was obtained. Doubly distilled lake water, testing P_H 6.3 to P_H 6.5 distilled first from an ordinary still and finally from a Bourdillon still with a block-tin condenser, was used in all of the experiments reported. A few crystals of potassium bisulphate were used in the Bourdillon still, as modified and described by Bennett and Dickson (*1*).

GLASSWARE

The highest-grade flasks, pipettes, and burettes were used throughout the investigation. All glassware was thoroughly seasoned in the beginning by being autoclaved in chromic-acid cleaning mixture for a period of one hour. The glassware was scrubbed with soap and hot water, soaked in chromic-acid cleaning mixture, washed repeatedly in tap water, rinsed in singly and in doubly distilled water, and protected from dust until needed.

EXAMINATION OF CULTURES

The experiments involving liquid media were conducted either in duplicate or in triplicate, and those involving solid media generally included 6 to 8 plates at each reaction. All cultures were inspected daily and records made accordingly. The growth data presented in this paper represent averages of these cultures. Dry weight of mycelium has been used as the criterion of growth in liquid media and diameter of colony for that in solid media. The substrate and fungous mat together were removed from each culture and filtered by suction through a Büchner funnel on a previously dried and weighed filter paper. The fungous material was washed several times with distilled water, the papers were then folded so as to prevent loss of material, and dried in an oven at 100° C. until a constant weight was obtained. An interval of from 24 to 48 hours was adequate. A definite time interval was allowed for the weighing of each empty filter paper and each filter paper containing fungous material. Where growth was very poor, as in Czapek's solution, much more consistent results were obtained for growth than when the papers were weighed without consideration of this point.

EXPERIMENTAL RESULTS

In presenting the results of the study, the data on the growth on solid media are given first, followed by similar data for the corresponding liquid media. The length of the incubation period and the temperature proved to be important factors with a direct bearing on the results obtained. The effects of these factors are considered subsequently under respective subheadings.

GROWTH ON SOLID MEDIA ADJUSTED TO DIFFERENT REACTIONS

In general, *Ophiobolus graminis* grew better on a solid substrate than in a liquid one. The rates of growth and the total quantities of growth within the favorable P_H ranges were more uniform for the several solid media tested than they were for the corresponding liquid media. Different criteria for growth necessarily were employed; that is, diameter of colony for the solid media and dry weight of the fungus for the liquid media, but the two methods were found to give more or less comparable results. The growth curves for the several solid media and the relations between the growth curves for the corresponding solid and liquid media will be discussed briefly.

The physical nature of the medium influenced the range of growth and the magnitude of growth quantities more in the case of potato dextrose than in any other. The range of growth was very restricted in potato-dextrose decoction, extending only from P_H 3.6 to P_H 7.7.

On potato-dextrose agar, however, the range expanded slightly on the acid side and markedly on the alkaline side, the growth range extending from P_H 3.2 to P_H 10.3. Two series of cultures on potato-dextrose agar were incubated in the dark at 24° C. The cultures were examined daily and remarkably consistent data were obtained, as shown by Table 2. A period of two days was necessary for growth to become measurable, and the growth increased and the limits expanded uniformly with increase of incubation period. At the end of seven days, growth occurred in all cultures between P_H 3.2 and P_H 9.6, and the colonies entirely filled the plates of cultures testing P_H 5.6 and P_H 5.9. Growth was very small in the most acid cultures, increased rather gradually to a maximum at P_H 5.6 to P_H 5.9, decreased to a minimum at P_H 9.2, and finally exhibited a tendency to increase in the most alkaline cultures. The growth curve for the second series is presented in Figure 1 with those from other solid media. The bimodal tendency was very pronounced and consistent in both series. An additional 6-day period of

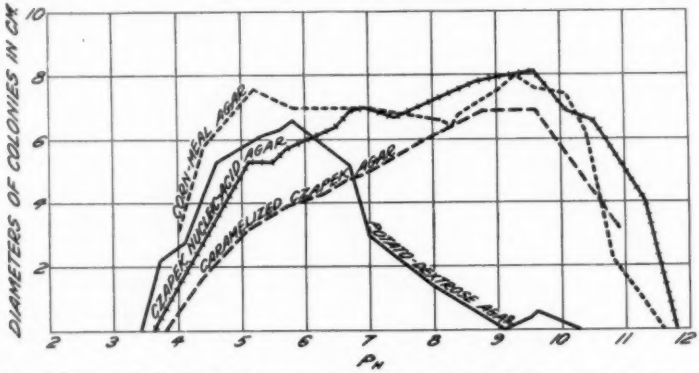

FIG. 1.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* on four solid media in a period of six days in the dark at 24° C.

incubation furnished very similar relations. The range of growth remained the same as before, i. e., P_H 3.2 to P_H 9.6; the bimodal tendency became more conspicuous, and growth covered the entire plates of those cultures testing P_H 4.8 to P_H 7.1 and P_H 9.6.

Caramelization of the potato-dextrose agar occurred in the most alkaline cultures and increased somewhat proportionally, as determined by the intensity of the brown coloration, with the quantity of alkali added. The stimulation of fungous growth seemed to be correlated more or less directly with this phenomenon up to a certain point. Cultures testing P_H 9.1 and P_H 9.2 were the most alkaline of these which did not show caramelization, and here the growth curve reached a minimum. Between the reactions of P_H 9.2 and P_H 10.3, the growth curve rose consistently and reached a maximum near P_H 9.5. Slight caramelization of the alkaline cultures in Czapek's solution likewise caused marked stimulation of fungous growth, whereas pronounced caramelization proved toxic to the fungus. Undoubtedly the conditions for growth in caramelized cultures are considerably different from those in noncaramelized cultures.

TABLE 2.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, on potato-dextrose agar for various periods in the dark at 24° C.*

FIRST SERIES

Hydrogen-ion concentration, P_H					Average diameter of colonies in centimeters after—														
Initial	After 7 days		Final		2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days
	Controls	Inoculated	Controls	Inoculated															
3.0	3.1	3.1	3.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0			
3.2	3.3	3.6	3.2	3.9	0	[a] F	F	0.7	0.8	0.9	1.3	1.6	1.8	1.9	2.3	2.4			
3.7	3.7	4.0	3.7	4.1	F	F	0.9	1.1	1.4	1.7	1.9	2.0	2.4	2.6	3.1	3.4			
4.1	4.1	4.3	4.1	4.3	F	1.2	1.9	2.4	3.5	3.7	4.1	4.6	5.3	5.6	6.5	7.1			
4.8	4.8	6.5	4.8	7.5	1.5	2.7	3.7	4.9	6.0	7.1	8.5	E	E	E	E	E			
5.3	5.3	8.5	5.4	8.1	2.0	3.3	4.6	5.7	7.8	8.7	[b] E	E	E	E	E	E			
5.6	5.6	8.7	5.6	8.1	2.2	3.4	4.7	6.0	8.1	E	E	E	E	E	E	E			
5.9	5.8	8.7	5.8	8.1	2.5	3.6	4.9	6.5	7.9	E	E	E	E	E	E	E			
6.6	6.6	8.6	6.5	8.3	2.2	3.5	4.5	5.9	7.4	8.3	E	E	E	E	E	E			
7.1	6.8	7.6		8.2	1.9	2.9	3.7	5.0	6.6	7.6	E	E	E	E	E	E			
8.2	7.6	7.6	7.4	8.2	F	1.0	1.5	2.0	2.8	3.5	4.4	5.3	6.9	7.4	8.1	8.7			
9.2	8.7	8.7	8.4	8.4	F	0.7	0.8	1.0	1.2	1.9	2.2	2.8	3.3	3.6	4.0	4.8			
9.5	8.9	8.9	8.7	8.6	F	0.7	1.3	1.9	2.8	3.6	4.3	5.4	6.4	7.2	8.1	8.7			
9.6	9.0	8.9	8.8	8.5	F	0.6	1.5	2.2	2.9	3.8	4.5	5.3	6.2	6.9	8.0	8.6			
9.6	9.0	8.9	8.8	8.6	F	1.0	1.7	2.3	3.3	4.0	4.8	5.8	6.9	7.7	8.6	E			
9.6	9.3	8.9	8.8	8.5	F	0.9	1.7	2.3	3.3	4.1	4.9	5.9	7.0	7.9	8.7	E			

SECOND SERIES

3.2	3.2		3.1	3.5	0	0	0	0	0	0	0	F	F	1.0	1.1	1.3	1.4	1.5	1.6
3.4	3.4		3.4	3.8	0	0	0	0	0	F	F	F	0.8	1.0	1.2	1.3	1.5	1.7	1.7
3.7	3.7	4.3	3.7	4.6	0	F	1.1	1.7	2.2	2.7	3.0	3.4	3.7	3.9	4.1	4.5	4.6	4.6	5.1
4.1	4.1	4.6	4.1	5.3	0	F	1.3	2.1	2.8	3.9	4.1	4.8	5.4	6.2	6.9	7.4	8.1	E	E
4.6	4.6	7.5	4.8	7.7	F	1.9	3.0	4.1	5.3	6.8	7.7	8.5	E	E	E	E	E	E	E
5.3	5.3	7.8	5.4	7.9	1.6	2.8	3.8	5.1	6.1	7.4	7.9	8.2	8.6	E	E	E	E	E	E
5.6	5.6	7.7	5.5	7.9	1.5	2.8	3.9	5.2	6.3	7.3	8.0	8.5	E	E	E	E	E	E	E
5.8	5.8	7.9	5.8	8.3	1.7	2.9	4.2	5.4	6.6	7.7	8.0	8.4	E	E	E	E	E	E	E
6.7	6.7	8.0	6.6	8.3	1.8	2.9	3.5	4.4	5.2	6.1	6.5	7.1	7.7	7.9	8.2	8.4	E	E	E
7.0	7.0	8.0	6.9	8.0	F	1.5	1.9	2.4	3.0	3.7	4.2	4.7	5.2	5.6	6.4	7.0	7.4	7.6	8.1
8.0	7.7		7.5	8.3	F	F	F	0.9	1.4	1.5	1.6	1.7	1.9	2.0	2.1	2.7	3.2	3.6	4.2
9.1	8.8		8.5	8.8	F	F	F	F	F	F	1.1	1.1	1.3	1.4	1.5	1.8	2.0	2.3	2.7
9.5	8.9		8.7	8.8	F	F	F	0.3	0.4	0.9	1.7	2.2	3.3	3.8	4.5	5.1	5.5	5.9	6.3
9.6	9.2		8.9	8.8	F	F	F	0.5	0.6	0.9	1.6	2.0	3.0	4.1	4.7	5.5	6.2	6.9	7.5
9.6	9.3	9.1	8.8	8.8	F	F	F	1.0	1.6	2.3	3.0	3.7	4.5	5.2	6.2	7.0	7.9	E	E
9.6	9.3	9.1	8.9	8.9	0	0	0	0	0	0.4	0.7	1.0	1.1	1.3	1.8	2.6	3.4	4.2	5.4
9.6	9.3	9.1	9.0	8.9	0	0	F	0.2	0.3	0.5	1.2	2.2	2.9	3.5	4.4	5.1	5.9	6.8	7.5
10.3	9.4		9.0	9.1	0	0	0	0	0	0	F	F	0.8	1.1	1.5	1.9	2.2	2.6	3.1

[a] F=trace of growth on edges of inoculum block.

[b] E=growth entirely covered 9-centimeter Petri dish.

Growth was very rapid on corn-meal agar at 24° C., and the fungus colonies covered the entire plates at the majority of the different initial reactions within a period of eight days. The results given in Table 3, and represented by one of the curves in Figure 1, show that the growth range extended to the limits of the experiment, i. e., P_H 4.0 to P_H 11.7, and that maxima occurred at P_H 5.2 and at P_H 9.3. A diminution in growth appeared at P_H 8.3 and this value for the corn-meal agar is very close to that furnished by the corn-meal decoction. This point may prove to be the isoelectric point of this medium, as determined by the growth of the fungus, but no definite statement can be made at this time. With six additional days of incubation, the range of best growth expanded and the colonies covered the entire surfaces of the cultures, ranging from P_H 4 to P_H 10.4. The most alkaline culture, possessing a reaction of P_H 11.7, was not far removed from the point of inhibition of growth. The most acid culture, i. e., P_H 4.0, did not even approach such a value.

The modified Czapek's full nutrient agar containing nucleic acid in place of $NaNo_3$ furnished interesting growth data, as shown by Table 4 and one curve in Figure 1. Growth on the solid medium was very good and much better than that in the corresponding liquid medium. The rate and the quantity of growth in the favorable cultures resembled that produced by potato-dextrose agar. Incubated at 24° C. in the dark for a period of seven days' growth occurred in all the cultures ranging from P_H 4.7 to P_H 11.3, and the colonies entirely covered the plates within the range of P_H 6.9 to P_H 10.1. Maximum growth, however, was obtained at P_H 9.6 on the sixth day of incubation, which was the day before the colonies covered the plates within the range mentioned. With prolonged intervals of incubation, the range of best growth expanded until it extended from P_H 4.7 to P_H 11.3 on the thirteenth day. No growth was obtained at P_H 3.0 or at P_H 11.8 and only slight growth at P_H 3.6.

Growth data from cultures on caramelized Czapek's agar (Table 4) resembled very closely those from cultures on Czapek's nucleic-acid agar. There is a striking similarity between the results of the two series, as shown by the growth curves of Figure 1. Because of the high acidity or of other chemical changes produced during the process of caramelization, the caramelized Czapek agar did not gel to the usual degree. Measurements of growth were particularly difficult to make under such conditions, especially as the fungus tended to grow less superficially and as the color contrast between fungous growth and medium was very slight. The range of growth extended from P_H 3.8 to the limit of the experiment, namely, P_H 10.9, and maximum growth occurred from P_H 8.8 to P_H 9.6+. The curve ascended to and descended from this conspicuous peak rather rapidly. The most acid cultures were incubated for an additional 5-day period, but the increase of growth in these cultures was remarkably small. These results may be found by reference to Table 4.

A considerable number of shifts in the reaction of the solid substrates were induced by the fungus during growth. In potato-dextrose agar, Table 2, the shifts were generally in the direction of alkalinity and the degree of the shift varied somewhat with the initial reaction. The changes were consistent for both series of potato-dextrose agar after 7 days' growth, but reversible shifts

TABLE 3.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, on corn-meal agar of different initial P_H values for various periods in the dark at 24° C.*

Hydrogen-ion concentration, P_H					Average diameter of colonies in centimeters after—												
Initial P_H	Final (7 days)		Final (14 days)		2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days
	Controls	Inoculated	Controls	Inoculated													
4.0	4.1	4.5	4.1	4.9	0.3	1.3	2.0	2.5	3.1	3.9	4.7	5.4	6.0	6.7	7.7	8.1	8.6
4.4	4.4	5.6	4.5	5.5	1.7	2.6	3.6	4.6	5.8	6.9	8.6	[a] E	E	E	E	E	E
5.2	5.3	7.0	5.2	6.8	1.7	3.1	4.6	6.0	7.5	E	E	E	E	E	E	E	E
5.8	5.8	7.3	5.5	7.2	2.1	3.1	4.5	5.8	7.0	8.4	E	E	E	E	E	E	E
6.8	6.8	7.6	6.9	7.4	2.0	3.0	4.4	5.6	7.0	8.5	E	E	E	E	E	E	E
7.1	7.2	7.7	7.2	7.5	1.9	3.0	4.3	5.6	6.9	8.3	E	E	E	E	E	E	E
7.5	7.5	7.9	7.9	7.9	1.7	3.0	4.2	5.4	6.8	8.0	E	E	E	E	E	E	E
8.1	8.0	8.1	8.2	8.2	1.8	2.9	4.2	5.5	6.6	8.9	E	E	E	E	E	E	E
8.3	8.3	8.3	8.4	8.3	1.6	2.8	4.0	5.2	6.4	7.9	E	E	E	E	E	E	E
8.4	8.5	8.4	8.6	8.4	1.8	2.9	4.2	5.5	6.8	8.3	E	E	E	E	E	E	E
9.0	8.9	8.7	8.9	8.7	1.7	3.0	4.7	6.0	7.5	E	E	E	E	E	E	E	E
9.3	9.0	8.8	9.2	8.9	1.7	3.4	4.8	6.3	8.0	E	E	E	E	E	E	E	E
9.6	9.1	8.9	9.3	9.2	1.8	2.9	4.4	5.9	7.6	E	E	E	E	E	E	E	E
10.1	9.2	9.1	9.4	9.3	1.7	3.0	4.4	5.9	7.4	8.7	E	E	E	E	E	E	E
10.4	9.3	9.3	9.5	9.4	1.4	2.5	3.7	5.0	6.2	7.2	8.4	E	E	E	E	E	E
10.8	9.5	9.5	9.6	9.5	[b] F	F	1.2	1.7	2.2	2.5	3.1	3.6	3.9	4.7	4.8	4.9	5.3
11.7	9.6	----------	9.6+	9.6	0	0	F	F	F	F	.5	.7	.9	.9	1.0	1.0	1.2

[a] E = growth entirely covered 9-0 centimeter Petri dish.

[b] F = trace of growth on edges of inoculum block.

TABLE 4.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, on Czapek's modified agar of different initial P_H values for various periods in the dark at 24° C.*

CZAPEK'S NUCLEIC-ACID AGAR

Hydrogen-ion concentration, P_H					Average diameter of colonies in centimeters after—											
Initial	After 7 days		After 17 days		2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days
	Control	Inoculated	Control	Inoculated												
2.3	.....	.....	.....	.....	0	0	0	0	0	0	0	0	0	0	0	0
2.5	.....	.....	.....	.....	0	0	0	0	0	0	0	0	0	0	0	0
3.0	3.1	.....	3.1	.....	0	0	0	0	0	0	0	0	0	0	0	0
3.6	3.6	.....	3.6	.....	0	0	[a] F	F	F	F	F	F	F	F	1.0	1.1
4.7	4.7	5.2	[b]	5.2	F	0.9	1.4	2.6	3.9	5.1	6.3	7.3	[c] E	E	E	E
5.1	5.2	5.6	5.1	5.6	F	1.8	3.0	4.2	5.3	6.3	7.8	E	E	E	E	E
5.5	5.5	5.8	5.6	5.7	F	1.6	2.9	4.1	5.3	6.6	7.9	E	E	E	E	E
5.7	5.6	5.7	[b]	5.8	F	1.7	3.4	4.6	5.7	7.1	E	E	E	E	E	E
6.5	6.5	6.6	6.4	6.6	0.9	1.9	3.4	4.9	6.4	7.8	E	E	E	E	E	E
6.7	6.7	6.7	6.6	6.8	1.6	2.1	3.9	5.2	6.9	8.3	E	E	E	E	E	E
6.9	6.9	6.9	6.9	7.0	1.2	2.3	3.9	5.2	7.0	E	E	E	E	E	E	E
7.4	7.2	7.2	7.1	7.2	1.1	2.2	3.9	5.3	6.7	E	E	E	E	E	E	E
8.7	8.3	8.1	8.3	8.7	1.2	2.8	4.4	6.1	7.8	E	E	E	E	E	E	E
9.6	8.7	8.4	8.7	8.3	1.2	2.9	4.8	6.3	8.1	E	E	E	E	E	E	E
10.1	8.8	8.7	8.9	8.7	.9	2.3	4.0	5.4	6.9	E	E	E	E	E	E	E
10.5	8.9	8.7	9.0	9.0	F	1.8	3.6	4.9	6.6	8.3	E	E	E	E	E	E
11.3	9.1	9.1	9.2	9.1	0	F	1.4	2.6	4.1	5.3	6.6	8.1	E	E	E	E
11.8	9.4	.....	9.5	.....	0	0	0	0	0	0	0	0	0	0	0	0

CZAPEK'S CARAMELIZED AGAR

3.1	.....	.....	.....	.....	0	0	0	0	0	0	0	.....	.....	.....	.....	.....
3.4	3.4	.....	.....	.....	0	0	0	0	0	0	0	.....	.....	.....	.....	.....
3.7	3.7	.....	.....	.....	0	0	0	0	0	0	0	0	0	0	0	0
3.8	3.8	.....	.....	.....	0	0	0	0	F	F	0.9	1.3	1.4	1.5	1.6	1.7
3.9	3.9	.....	.....	.....	0	F	F	F	0.3	0.3	.6	1.2	1.4	1.7	2.0	2.1
4.3	4.3	4.4	.....	.....	0	F	0.6	1.3	1.5	1.9	2.3	2.4	2.4	2.9	2.9	.....
5.1	5.1	5.2	.....	.....	0	0.2	1.7	2.2	3.2	3.4	3.6	.....	.....	.....	.....	.....
5.7	5.7	5.4	.....	.....	0.2	.8	2.7	3.1	3.9	5.0	5.1	.....	.....	.....	.....	.....
6.3	6.2	5.7	.....	.....	0	F	2.6	3.5	4.3	5.1	5.6	.....	.....	.....	.....	.....
6.6	6.5	6.2	.....	.....	0	0	2.9	4.0	4.7	5.7	6.1	.....	.....	.....	.....	.....
6.9	6.8	6.6	.....	.....	0	0	2.5	4.0	4.9	5.8	6.1	.....	.....	.....	.....	.....
7.8	7.2	7.1	.....	.....	0	0	3.8	5.0	5.9	7.0	8.0	.....	.....	.....	.....	.....
8.8	7.5	7.5	.....	.....	.8	1.6	4.1	5.7	6.9	7.7	8.6	.....	.....	.....	.....	.....
9.5	8.1	8.1	.....	.....	1.7	3.1	3.9	6.3	6.9	8.4	E	.....	.....	.....	.....	.....
9.6+	8.6	8.4	.....	.....	1.7	2.8	3.8	5.7	6.9	8.5	E	.....	.....	.....	.....	.....
9.6+	8.6	8.6	.....	.....	1.5	2.6	3.5	5.2	6.2	7.3	7.7	.....	.....	.....	.....	.....
10.9	8.6	8.6	.....	.....	F	1.1	1.6	2.5	3.2	3.7	5.1	5.4	.....	.....	.....	.....

[a] F=trace of growth on edges of inoculum block. [b] Cultures contaminated. [c] E=growth entirely covered 9-centimeter dish.

appeared after 14 days' growth in the acid cultures of the first series which exhibited best growth. Growth was generally less in the second series than in the first series. The cultures either maintained their reactions or became more alkaline with prolonged incubation, and there were no indications of reversible shifts. The degree of the secondary shift in potato-dextrose agar was smaller as compared to that in potato-dextrose decoction. Buffer action is much stronger in potato-dextrose agar than in potato-dextrose decoction, and this may assist in explaining the variations.

Corn-meal agar, as influenced by growth of the fungus, showed changes in reaction of the medium somewhat similar to those of potato-dextrose agar. The reaction of the acid cultures shifted towards alkalinity and attained an alkaline value in many cases, whereas that of the alkaline ones either remained constant or became slightly less alkaline. Final determinations of the most alkaline uninoculated controls showed a considerable diminution of alkalinity upon standing seven days. These results are included in Table 3.

The fungus produced very slight changes or none at all in the Czapek agar containing nucleic acid and in the caramelized Czapek agar. Marked changes occurred in the reaction of the most alkaline inoculated cultures, but the changes were no greater than those in the corresponding uninoculated controls. Table 4 contains the data for the caramelized and the nucleic-acid Czapek agar.

TABLE 5.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, in potato-dextrose decoction of different initial P_H values, temperatures, and periods of incubation in the dark*

Incubation		Hydrogen-ion concentration, P_H			Dry weight of fungous mat
			Final		
Temperature, °C.	Time in days	Initial	Control	Inoculated	
					Gm.
10	27	2.9	2.9	2.9	0.0000
		3.9	3.8	4.9	.0664
		4.9	5.0	7.0	.1456
		5.8	5.8	7.4	.1219
		6.7	6.7	7.3	.1382
		7.1	6.9	7.4	.1281
		7.5	7.4	7.8	.1002
		8.4	8.2	7.9	.0108
		8.8	8.5	8.5	.0000
24	12	2.9	2.9	2.9	.0000
		3.9	4.0	4.4	.1378
		4.9	4.9	7.2	.2020
		5.7	5.6	7.3	.1971
		6.5	6.4	7.2	.1880
		6.9	6.8	7.3	.1758
		7.4	7.2	7.3	.2123
		8.3	8.1	8.1	.0000

GROWTH ON LIQUID MEDIA ADJUSTED TO DIFFERENT REACTIONS

The results indicate that the influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* varies considerably, and depends to a large extent upon the nutritive relations and other factors. The organism is capable of growing throughout a wide range of hydrogen-ion concentrations in all media employed, except potato-dextrose decoction. Referring to Tables 5 and 6 and to

Figures 2, 3, and 4 it will be seen that on potato-dextrose decoction the fungus had a relatively restricted range, i. e., P_H 3.6 to P_H 7.7. Despite the fact, however, that such a narrow range for growth occurred in this medium, it consistently produced excellent growth at the favorable reactions, and therefore is regarded as the best medium employed in this study.

TABLE 6.—*The growth of Ophibolus graminis, and the changes in reaction induced by growth, in potato-dextrose decoction in diffused light at different temperatures, periods of incubation, and initial P_H values*

Incubation		Hydrogen-ion concentration, P_H			Dry weight of fungus mat
Temperature, °C.	Time in days	Initial	Final		
			Control	Inoculated	
					Gm.
10	12	2.9	2.8	2.8	0.0000
		3.9	3.8	4.0	.0077
		4.9	4.9	5.1	.0100
		5.8	5.8	6.1	.0114
		6.7	6.6	6.9	.0169
		7.1	6.9	7.1	.0078
		7.5	7.4	7.1	.0070
		8.4	8.2	8.0	.0000
16	12	2.9	3.0	2.9	.0000
		3.6	3.5	3.6	.0007
		4.1	4.1	4.4	.0243
		4.9	5.0	5.7	.0357
		5.8	5.9	6.6	.0416
		6.4	6.5	6.8	.0529
		6.8	6.7	7.0	.0565
		7.2	7.0	7.1	.0617
		7.7	7.4	7.4	.0091
		8.4	8.2	8.0	.0000
20	12	2.9	2.9	2.9	.0000
		3.6	3.5	3.6	.0065
		4.1	4.1	5.0	.0811
		4.9	4.9	6.6	.0717
		5.8	6.1	7.2	.0841
		6.4	6.2	7.3	.1226
		6.8	6.6	7.6	.1405
		7.2	7.0	7.6	.1377
		7.7	7.3	7.3	.0107
		8.4	8.1	8.0	.0000
24	8	2.9	3.0	3.0	.0000
		3.6	3.5	3.5	.0027
		4.1	4.1	4.4	.0326
		4.9	4.8	5.3	.0367
		5.8	5.7	6.3	.0471
		6.4	6.4	6.7	.0577
		6.8	6.8	6.9	.0584
		7.2	7.1	7.2	.0723
		7.7	7.4	7.4	.0035
		8.4	8.3	8.2	.0000
24	12	2.9	2.9	2.9	.0000
		3.9	4.1	4.7	.1053
		4.9	5.0	7.3	.1959
		5.7	6.1	7.4	.2021
		6.5	6.8	7.5	.1985
		6.9	6.8	7.5	.1884
		7.4	7.4	7.6	.1914
		8.3	8.1	8.1	.0000
28	12	2.9	2.9	2.9	.0000
		3.6	3.6	3.6	.0000
		4.1	4.1	3.9	.0114
		4.9	5.0	5.2	.0148
		5.8	5.8	5.9	.0344
		6.8	6.4	6.8	.0515
		7.2	6.8	6.8	.0228
		7.7	7.3	7.4	.0116
		8.4	7.9	----------	.0000

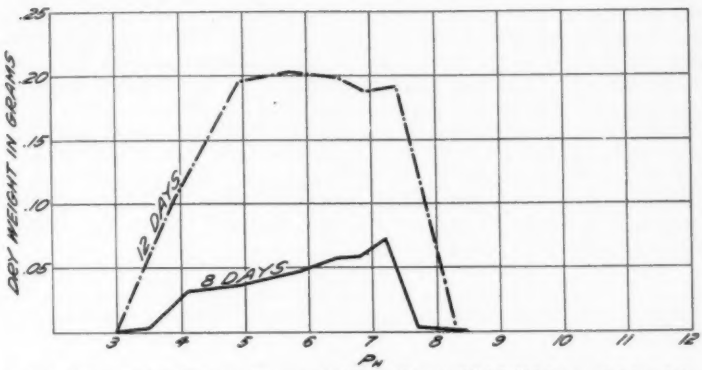

FIG. 2.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in potato-dextrose decoction for 8 and 12 days in diffused light at 24° C.

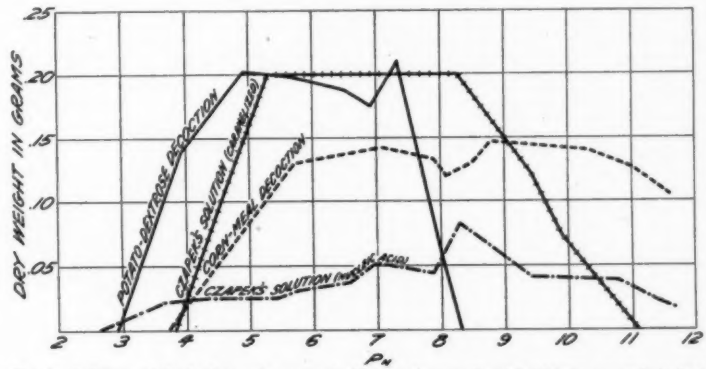

FIG. 3.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in four liquid media in a period of 12 days in the dark at 24° C.

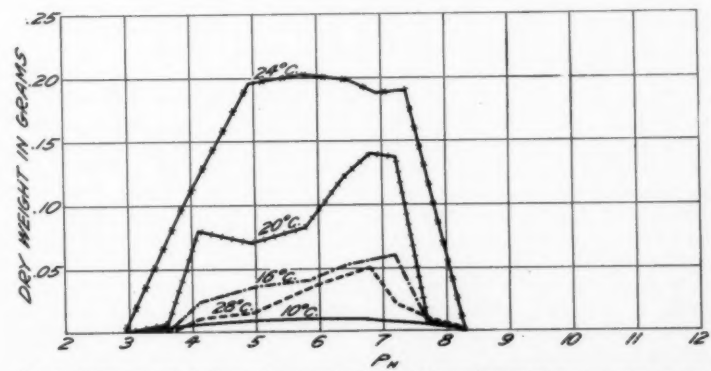

FIG. 4.—Influence of the hydrogen-ion concentration on the growth of *Ophiobolus graminis* in potato dextrose decoction at different temperatures for a period of 12 days in diffused light

A corn-meal decoction, as shown by Table 7, furnished growth at concentrations from P_H 3.6 to P_H 12.1. Two series of experiments were conducted in the dark for 12 days at 24° C. The first series produced only from one-third to one-fifth as much growth, on the average, as the second. Figure 3 contains the growth curve for the second series. Such uniform variation throughout the growth range might perhaps be explained on a basis of vitality of the culture which served for the inoculum. Similar variations have occurred for different series with a particular medium. A corn-meal decoction is a poorly buffered medium, and for this reason it is rather unsatisfactory for this type of experimentation.

TABLE 7.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, in corn-meal decoction at different initial P_H values in the dark for 12 days at 24° C.*

Hydrogen-ion concentration, P_H			Dry weight of fungous mat	Hydrogen-ion concentration, P_H			Dry weight of fungous mat
Initial	Final			Initial	Final		
	Control	Inoculated			Control	Inoculated	
			Gms.				*Gm.*
3.2	3.0	3.0	0.0000	3.3		3.4	0.0000
3.3	3.2	3.2	.0000	3.7	3.6		* F
3.6	3.6	4.1	.0117	5.7	5.6	6.5	.1308
4.3	5.6	5.8	.0316	7.1	6.0	6.8	.1432
6.9	6.0	6.7	.0358	7.9		7.7	.1331
8.1	7.9	7.3	.0277	8.1	7.5	7.3	.1192
8.2	8.3	7.6	.0357	8.5		7.4	.1304
8.7	8.4	7.9	.0383	8.8	8.4	7.7	.1469
10.4	8.9	8.4	.0340	10.3	8.9	8.1	.1406
11.5	9.3	9.0	.0217	11.0		8.7	.1263
12.1	9.5	9.4	.0161	11.6	9.3	9.0	.1048
12.2	9.6	9.6	.0000				

* F=trace of growth on edges of inoculum block.

Ophiobolus graminis grew feebly on the ordinary Czapek's solution and variably on Czapek's solution modified by the substitution of nucleic acid for sodium nitrate, as shown by Table 8. In the ordinary Czapek's solution at 24° C., growth occurred throughout the range P_H 4.5 to P_H 10.5. Greatest growth occurred at P_H 7.0 and at P_H 8.0, with an intervening minimum at P_H 7.6. In the nucleic-acid Czapek's solution, the reactions permitting growth ranged from P_H 3.6 to P_H 11.7, and the maximum growth of the fungus occurred in the cultures testing P_H 8.3. Reference to Figure 3 will reveal these relations. The hydrogen-ion concentration necessary to limit the growth on the alkaline side lay beyond the range of the experiment, i. e., P_H 11.7. Such also was the case with the corn-meal decoction. Growth was slightly better in the nucleic-acid Czapek medium than in the ordinary Czapek medium. In a preliminary series containing nucleic acid and adjusted to P_H 7.0, growth was highly stimulated. Definite and well-formed mats occurred in all of the cultures and the growth resembled that on potato-dextrose decoction.

TABLE 8.—*The growth of Ophiobolus graminis, and the changes in reaction induced by growth, in ordinary and modified Czapek's solutions at different initial P_H values for various periods in diffused light and in the dark at 24° C.*

Media	Incubation	Hydrogen-ion concentration, P_H			Dry weight of fungous mat
		Initial	Final		
			Control	Inoculated	
					Gm.
Czapek's solution	29 days in diffused light.	2.6	2.6	2.7	0.0000.
		3.0	3.0	3.0	0.0000.
		3.4	3.5	3.5	0.0000.
		4.5	4.5	3.9	0.0092.
		5.6	5.5	4.5	0.0051.
		6.6	6.4	6.5	0.0268.
		7.0	6.8	7.2	0.0674.
		7.6	7.6	6.2	0.0214.
		8.0	8.0	7.6	0.0546.
		10.5	9.0	9.0	0.0093.
Czapek's solution (with nucleic acid).	12 days in dark	2.6	2.7	2.6	0.0000.
		3.6	3.0	3.6	0.0218.
		4.4	----	4.4	0.0257.
		5.4	5.4	5.1	0.0251.
		5.7	5.7	5.3	0.0298.
		6.6	6.4	6.4	0.0384.
		7.0	6.8	6.6	0.0527.
		7.9	7.2	6.8	0.0459.
		8.3	7.5	7.4	0.0836.
		9.4	8.6	8.2	0.0419.
		10.8	9.0	8.6	0.0381.
		11.4	9.1	9.1	0.0220.
		11.7	9.3	9.4	0.0166.
Czapek's solution (caramelized strongly).	41 days in dark	3.6	3.4	----	0.0000.
		4.7	3.9	----	0.0138.
		5.5	4.9	5.2	0.0262.
		5.8	5.3	5.3	0.0301.
		6.5	6.0	5.6	0.0319.
		6.8	6.5	5.8	0.0293.
		7.3	6.8	6.5	0.0271.
		9.2	7.2	7.0	0.0271.
		9.6	7.4	7.1	0.0238.
		10.2	7.6	7.4	0.0204.
		10.7	8.7	8.2	0.0299.
		11.0	9.0	8.6	0.0298.
Czapek's solution (caramelized slightly).	12 days in dark	3.6	----	----	No growth.
		3.8	----	----	Trace.
		5.3	----	----	Heavy mat.
		5.9	----	----	Do.
		6.8	----	----	Do.
		7.5	----	----	Do.
		7.9	----	----	Do.
		8.3	----	----	Do.
		9.4	----	----	Medium mat.
		9.9	----	----	Growth throughout, no mat.
		10.7	----	----	Slight growth.
		11.1	----	----	No growth.

It was repeatedly noticed that several flasks from each lot of Czapek's solution possessed a faint brownish tint. This condition occurred even though the acid and alkali were sterilized separately and subsequently added to the various cultures. The phenomenon of caramelization is extremely complicated and is understood only in a general way. In this connection, it must be remembered that the initial reaction of Czapek's solution generally tested P_H 4.6 and that the carbohydrate employed was sucrose. Such conditions are very conducive to caramelization. Heavy fungous mats, similar to those formed in potato-dextrose decoction, were produced in caramelized cultures of Czapek's solution as contrasted with the

small fungous colonies produced in the noncaramelized cultures. A preliminary series of experiments was conducted for a period of 12 days at 24° C. with caramelized Czapek's solution adjusted to various initial reactions. The results are presented in Table 8. Growth was obtained within the range of P_H 5.3 and P_H 10.7 with heavy fungous mats produced in cultures testing between P_H 5.3 and P_H 8.3 inclusive. In this very preliminary series no dry weights and no final hydrogen-ion concentrations of the substrate were determined. The curve for caramelized Czapek's solution in Figure 3 was approximated from the results of this series.

Incubated under similar conditions, very slight growth occurred in the strongly caramelized solutions throughout the entire range, i. e., P_H 4.7 to P_H 11.0, as shown by Table 8. This uniformly poor growth can not be attributed to the viability of the inoculum, inasmuch as the cultures were reinoculated 10 days after the first inoculation. The treatment given the solutions evidently was too drastic to permit growth of the fungus. In this case, it will be remembered that the concentrated nutrient solution (10X) was caramelized by autoclaving for 4 hours at a 15-pound pressure as compared with 2 hours at a 15-pound pressure in the other. These solutions, as determined by the intensity of the amber color, were caramelized to a much higher degree than those of the few slightly caramelized cultures appearing in the ordinary series, or of the slightly caramelized cultures in the preliminary series. Whereas a slightly caramelized Czapek's medium stimulated growth, a strongly caramelized Czapek's medium proved toxic to the fungus. A similar relationship was noted for varying degrees of caramelization in the alkaline cultures of potato-dextrose agar.

In the studies with *Ophiobolus graminis*, the final hydrogen-ion determinations showed that the fungus generally induced a shift in reaction toward alkalinity in a potato-dextrose medium, shifts toward acidity in Czapek's nucleic-acid and Czapek's ordinary media, and a shift toward neutrality in a corn-meal decoction. There appeared early in the work, however, indications that the changes in the liquid media did not always continue in their original direction.

For the studies of progressive growth, several series of experiments were conducted with potato-dextrose decoction adjusted to different initial reactions, namely, P_H 4.3, the most acid reaction allowing good growth; P_H 5.5, a potato-dextrose decoction without additions of either acid or alkali, and P_H 6.5, the reaction near the alkaline limit affording good growth. The necessary number of inoculated and uninoculated flasks were placed in the dark at 24° C. Three inoculated cultures and one uninoculated control were removed every two days. Dry weights of the fungous mats and the hydrogen-ion concentrations of inoculated and uninoculated media were determined at each periodic removal. The progressive relations for the growth of the fungus and for the changes in reaction of the medium are presented by data in Table 9, and by curves in Figures 5 and 6. At first, growth was more rapid in the nearly neutral cultures; later, it was greater in the more acid cultures. In general, growth increased with the incubation interval, and the greatest increase of growth occurred during the last two days of the experiment, namely, between 18 and 20 days.

TABLE 9.—*The progressive growth of Ophiobolus graminis, and the progressive changes in reaction induced by growth, in potato-dextrose decoction in the dark for various periods at 24° C.*

Age of cultures	Dry weight of material	Hydrogen-ion concentration, P_H		
		Initial	Final	
			Control	Inoculated
Days	*Gm.*			
2	0.0036	4.3	4.3	4.3
4	.0114	4.3	4.3	4.3
6	.0237	4.3	4.3	4.4
8	.0959	4.3	4.3	5.1
10	.1756	4.3	4.3	5.7
12	.1820	4.3	4.3	5.4
14	.2598	4.3	4.3	5.4
16	.2384	4.3	4.3	4.9
18	.2636	4.3	4.3	4.9
20	.5194	4.3	4.3	4.9
2	0.0019	5.5	5.5	5.5
4	.0151	5.5	5.5	5.5
6	.0236	5.5	5.5	5.7
8	.0859	5.5	5.5	6.7
10	.1478	5.5	5.5	7.8
12	.1908	5.5	5.5	6.9
14	.2079	5.5	5.5	6.8
16	.1522	5.5	5.5	5.7
18	.2340	5.5	5.5	5.5
20	.4340	5.5	5.5	4.9
2	0.0035	6.5	6.5	6.5
4	.0155	6.5	6.5	6.6
6	.0267	6.5	6.5	6.6
8	.1048	6.5	6.5	7.2
10	.1288	6.5	6.5	7.4
12	.1627	6.5	6.5	7.0
14	.1814	6.5	6.5	5.8
16	.1770	6.5	6.4	5.5
18	.2103	6.5	6.4	5.4
20	.3898	6.5	6.3	5.2

The changes in reaction of the potato-dextrose decoction during growth of the fungus were very definite and similar for each of the initial reactions, as shown by Table 9 and Figure 6. The uninoculated control cultures held their initial reaction throughout the 20 days of the experiment, and they will not be considered further. At P_H 4.3, the acidity of the medium decreased with increased growth until a value of P_H 5.7 was reached on the tenth day. After this date, even though growth continued to increase, the acidity increased until the final reaction tested P_H 4.9 for 20 days of fungous growth. A distinct reversion in reaction of the medium occurred on the tenth day, and the final reaction was six-tenths of an exponent less acid than that representing the initial reaction. Similar but greater changes were obtained at P_H 5.5. The reaction of the substrate progressively changed until a value of P_H 7.8 was reached on the tenth day. After this point, a reversion in reaction manifested itself, progressive changes occurred, and P_H 4.9 was the final reaction recorded. It is interesting to note that the cultures possessed a final value six-tenths of an exponent more acid than they did originally. Very similar changes were obtained for the cultures initially testing P_H 6.5. The changes increased successively until the tenth day was reached and a value of P_H 7.4 exhibited. After this time the changes proceeded

in the opposite direction and the final hydrogen-ion concentration was P_H 5.2. This value is actually one and three-tenths of an exponent more acid than originally. The greatest changes in reaction induced by growth occurred between the sixth and the sixteenth days of incubation and, of the three initial reactions employed, namely, P_H 4.3, P_H 5.5, and P_H 6.5, the greatest changes in reaction of the substrate occurred at P_H 5.5.

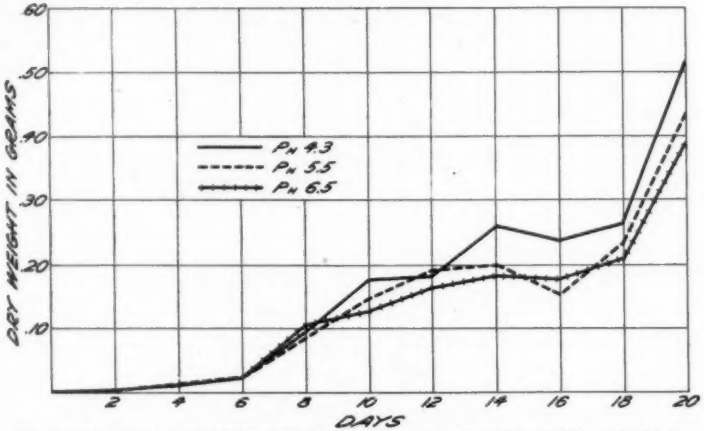

FIG. 5.—The growth of *Ophiobolus graminis* in potato-dextrose decoction of different initial P_H values for various periods in the dark at 24° C.

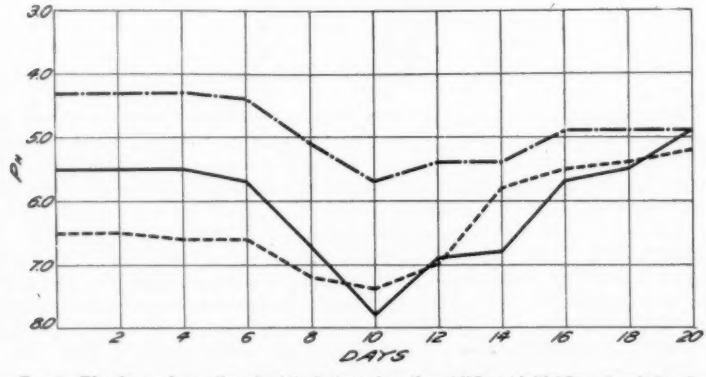

FIG. 6.—The changes in reaction of potato-dextrose decoction of different initial P_H values induced by the growth of *Ophiobolus graminis* for various periods in the dark at 24° C.

Similar progressive series of experiments were conducted with Czapek's full nutrient solution modified by the substitution of nucleic acid for $NaNO_3$ and adjusted to initial values of P_H 4.9 and of P_H 8.3. The results are shown in Table 10 and in Figures 7 and 8. Very little growth was obtained at P_H 4.9, and it was more or less constant after an incubation of six days. Growth was much better at P_H 8.3, and increased rather uniformly with increase in

time interval. The reactions of the cultures at different ages showed reversion phenomena, but they were in the opposite direction to those occurring in potato-dextrose decoction. In the cultures initially testing P_H 4.9, the changes were relatively small. The acidity of the substrate increased with increase in age of the culture until a value of P_H 4.2 was reached on the fourteenth day. Two days later, the reaction became P_H 4.4, and remained constant throughout the duration of the experiment. Similar changes of

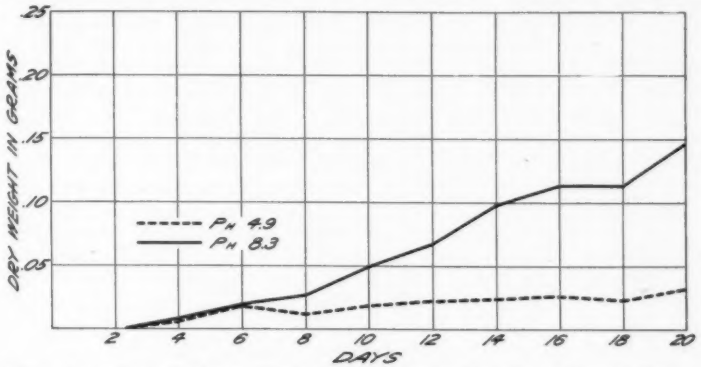

FIG. 7.—The growth of *Ophiobolus graminis* in Czapek's nucleic-acid solution of different initial P_H values for various periods in the dark at 24° C.

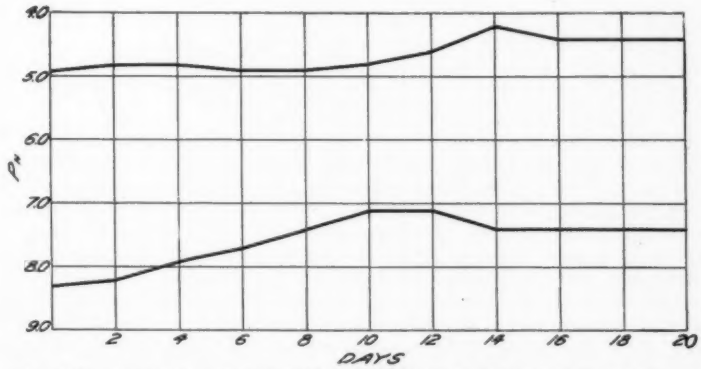

FIG. 8.—The changes in reaction of Czapek's nucleic-acid solution of different initial P_H values induced bythe growth of *Ophiobolus graminis* for various periods in the dark at 24° C.

greater magnitude occurred in the cultures testing P_H 8.3. The alkalinity of the substrate decreased with increased period of incubation and attained its lowest value, namely, P_H 7.1 for both 10 and 12 days' growth. The reaction shifted to P_H 7.4 after two additional days of growth, and subsequent determinations proved it to be constant for the remainder of the experiment. The changes were first in the direction of the acid side and then in the direction of the alkaline side. Opposite shifts, on the other hand, appeared with potato-dextrose decoction. In the Czapek medium, the magnitude

of the second shift did not equal that of the initial shift. Such a relation held true for the potato-dextrose decoction only at an initial P_H 4.9, whereas at initial reactions of P_H 5.5 and P_H 6.5 the reversion shifts were greater than the initial shifts.

TABLE 10.—*The progressive growth of Ophiobolus graminis, and the progressive changes in reaction induced by growth, in Czapek's nucleic-acid solution in the dark for various periods at 24° C.*

Age of cultures	Dry weight of material	Hydrogen-ion concentration, P_H		
		Initial	Final	
			Control	Inoculated
Days	*Gm.*			
2	[a] Tr.	4.9	4.9	4.8
4	0.0077	4.9	4.8	4.8
6	.0218	4.9	4.9	4.9
8	.0132	4.9	4.9	4.9
10	.0196	4.9	4.8	4.8
12	.0230	4.9	4.8	4.6
14	.0252	4.9	4.8	4.2
16	.0274	4.9	4.8	4.4
18	.0229	4.9	4.8	4.4
20	.0318	4.9	4.8	4.4
2	Tr.	8.3	8.3	8.2
4	.0106	8.3	8.3	7.9
6	.0219	8.3	8.3	7.7
8	.0285	8.3	8.4	7.4
10	.0503	8.3	8.3	7.1
12	.0688	8.3	8.3	7.1
14	.0982	8.3	8.4	7.4
16	.1134	8.3	8.3	7.4
18	.1124	8.3	8.3	7.4
20	.1458	8.3	8.3	7.4

[a] Tr. = trace of growth.

In order that it might be determined whether growth had merely been inhibited or the fungus had actually been killed in the extremely acid and alkaline cultures not showing growth, the inoculum blocks were transferred from such cultures to potato-dextrose agar slants at the termination of several experiments with potato-dextrose decoction and with Czapek's solution. In potato-dextrose decoction, it will be remembered, growth occurred throughout the range P_H 3.9 to P_H 7.7, and no growth occurred in the acid cultures testing P_H 2.4, 2.9, and 3.6, or in the alkaline cultures testing P_H 8.4, 8.6, 8.8, and 9.5. The results obtained from the transfers show that the fungus was killed at the reactions P_H 2.4 and 9.5 and that it was still alive at the reactions P_H 2.9, 3.6, 8.4, 8.6, and 8.8 even though no perceptible growth had occurred at these reactions for a period of 12 days. Very consistent results were obtained at all the reactions except P_H 2.9, where probably not more than 50 per cent of the transfers grew. This indicated that such a reaction was very close to the critical one for the fungus in this medium. Somewhat similar but variable results were obtained with the transfers from Czapek's solution.

When acid or alkali in sufficient quantities to neutralize the acid or alkali originally employed for the adjustment of the reactions were added to the cultures of potato-dextrose decoction not showing

growth, no perceptible growth was ever exhibited. Subsequent transfers of the inoculum blocks were not made, but it would seem that nutritional disturbances produced by the second addition of either acid or alkali were responsible for either inhibition of growth or death of the fungus.

EFFECT OF LENGTH OF INCUBATION PERIOD

The correlation between the growth of *Ophiobolus graminis* and the hydrogen-ion concentration of the medium is influenced to a considerable degree under certain conditions by the length of the incubation period. In potato-dextrose decoction, growth generally occurred first and most rapidly at or near neutrality. However, with continued incubation, the rate of growth and the total quantity of growth in the acid cultures surpassed that in the approximately neutral ones. Different types of growth curves, therefore, were obtained when cultures were incubated under the same conditions for various periods. The growth curves in Figure 2 for two potato-dextrose decoction series at 24° C., one for a period of 8 days and the other for a period of 12 days, illustrate this point.

For the 8-day series, the growth curve gradually rose from P_H 3.6, reached a distinct maximum at P_H 7.2, and declined rapidly to P_H 7.7. The 12-day series, on the other hand, produced a rather flat type of growth curve with maximum growth occurring rather uniformly throughout a zone of reactions, namely, P_H 4.9 to P_H 7.4. This curve obtained for 8 days' growth at 24° C. is almost identical with the one obtained for 12 days' growth at 16°, and very similar to those obtained for 12 days' growth at 20° and 28°. At 10° growth was very small and more or less uniform throughout the growth range for a period of 12 days. An additional 15-day period of incubation at this temperature gave a bimodal growth curve with the primary maximum occurring at P_H 4.9 and the secondary one near P_H 7.0. Growth occurred from P_H 3.9 to P_H 8.4 and the range was slightly wider than usual, the alkaline limit having shifted a few tenths of a P_H toward greater alkalinity. The length of time necessary for the fungus to stale potato-dextrose decoction, 50 c. c. portions per culture, and thus cease growing has not been determined. Experiments were conducted over a period of 20 days and cultures were removed every two days. Growth continued throughout the entire period and occurred most rapidly for each of the three initial reactions near the end of the experiment. The data for potato-dextrose decoction are assembled in Tables 5, 6, and 9, and are graphically represented by curves in Figures 2, 3, 4, and 5.

Czapek's nutrient solution, modified by the substitution of nucleic acid for sodium nitrate, gave different results from those furnished by potato-dextrose decoction. In the acid cultures, feeble growth appeared early, but it never increased to any perceptible degree after the sixth day. Good growth, on the other hand, was obtained in the alkaline cultures and it increased more or less uniformly with increased periods of incubation. These results are included in Table 10 and serve as a basis for the curves in Figure 7.

With the corresponding solid media, growth occurred first and most rapidly at certain initial reactions, depending upon the medium, and the relations which were established early generally persisted throughout the course of the experiment. The fungous colonies were meas-

ured daily and very consistent data were obtained for the several media employed. At the optimum reactions, a period of 6 to 7 days was generally necessary to enable the fungous colonies entirely to cover the plates. The cultures were allowed to incubate over a period of 12 to 15 days and, while it is not possible to say anything definitely about the later growth at the favorable reactions, accurate growth data were obtained for the other reactions until the fungous colonies completely covered the plates.

Simultaneous with the studies on progressive growth in potato-dextrose decoction and Czapek's modified solution, progressive changes in reactions of the substrate induced by growth of the fungus were likewise followed. The occurrences of progressive and reversible shifts in reaction during the growth of this organism detract more or less from any significance that might be attached to the final hydrogen determinations and emphasize the importance of following such changes in reaction of the medium during growth.

EFFECT OF TEMPERATURE AND LIGHT

Like the chemical composition and the physical nature of the medium, temperature influenced the growth of the fungus, as related to hydrogen-ion concentration of the medium. Within the limits of the experiments, temperature did not materially alter the P_H range within which growth occurred, but it did influence the magnitude of growth at various initial reactions. Inasmuch as potato-dextrose decoction produced the best fungous growth, the temperature studies were confined principally to this medium. A few experiments, however, were conducted with the Czapek's synthetic solution, but growth in this medium was so poor that the results are not satisfactory.

Incubated for a period of 12 days in diffused light, the fungus grew best in potato-dextrose decoction at 24° C., followed in order by 20°, 16°, 28°, and 10°. The data presented in Table 6 and represented graphically in Figure 4 reveal these relations. At the optimum temperature, the growth range extended from P_H 3.6 to P_H 7.7, with best growth occurring more or less uniformly between P_H 4.9 and P_H 7.4. The growth range was practically the same for each of the other temperatures. The alkaline limit remained the same, and the acid limit at 28° and 16°, shifted several tenths of an exponent toward neutrality. At 24° growth in the extreme cultures was good, but at the other temperatures it was very small, in fact almost immeasurable in certain instances. Maximum growth of the fungus occurred in the form of a peak at or near P_H 7.0 for temperatures of 20°, 16°, and 28°, as contrasted with a broad zone from P_H 4.9 to P_H 7.4 for 24°. Heavy fungous mats were produced in all of the cultures at 24° and in those with favorable reactions at 20°. Growth was much less at 16° and 28°, and there were either very thin fungous mats or no mats at all. At 10°, mycelial growth was exceedingly slight and uniform, an interval of seven days being necessary before a trace of growth could be detected around the inoculum block. The final colonies were thin, flat, grayish, and filmlike.

Light is frequently regarded as an important factor for the fruiting of certain fungi, but neither perithecia nor indications of such appeared during the course of the experiments reported in this paper. Davis (*4*) has stated that diffused light slightly retarded mycelial development of *Ophiobolus graminis* in pure culture. In our studies,

however, diffused light did not seem to exert any appreciable effect on the relation of vegetative growth to the reaction of the medium.

Two series of experiments were conducted with potato-dextrose decoction at 24° C., one in the dark and one in diffused light. The growth curves, Figures 3 and 4, developed from the two experiments are very similar. There was a slightly greater tendency for a bimodal-growth curve in the case of the series incubated in the dark than in the one in the light. The variations in the two growth curves, however, were not very great and the P_H growth ranges were the same in both cases. The fungous mats obtained from the series grown under diffused light were thick, heavy, and substantial. They adhered strongly to the sides of the flasks and always remained in perfect form at the surface of the liquid medium. On the other hand, the fungous mats obtained from the series incubated in the dark were equally as thick and heavy, but were less firmly attached to the sides of the flasks. These mats generally became either completely or incompletely detached from the flasks and consequently appeared partially or totally submerged in the culture solutions. More or less consistent results were obtained for potato-dextrose decoction in the light and in the dark at 10°, and for Czapek's unmodified solution in the light and in the dark at 24° and 28°. In view of these consistencies, the tabulated results of the experiments for each series in the light and in the dark have not been included.

DISCUSSION

The results obtained by the writers show that the influence of the hydrogen-ion concentration on the vegetative growth of *Ophiobolus graminis* in pure culture is variable, and depends to a large extent on the chemical composition and the physical nature of the medium. The P_H ranges for fungous growth may be either wide or narrow, more generally the former, and the growth curves may be either monomodal or bimodal. Furthermore, the optimum P_H for fungous growth may fall either in the distinctly acid or the alkaline regions, or it may span the intervening neutral zone. It appears from these facts, therefore, that the general conclusions drawn by Kirby (*7*, *8*) and Davis (*4*), regarding the influence of the hydrogen-ion concentration on the growth of the parasite in pure culture, are valid only within the limits of their experiments.

Brittlebank (*2*), who has investigated the take-all problem for a number of years in Australia, frankly admits that very little is known concerning the influence of soil reaction on the development of the disease. From the results obtained by him in one year's study on the permanent test plats, it appears that where lime in combination with other fertilizers had been applied on alternate years during a period of six years, the disease was far more prevalent than where lime had been omitted. This correlation refers more to the percentage of diseased plants than to the severity of the disease. Brittlebank says: "It would appear, therefore, that an alkaline soil is favorable to the development of the disease, but it should be again remarked, this judgment is from one year's data only." Another statement under the same heading, namely, "it is not known whether the fungus favors an acid or an alkaline soil, or one rich in organic matter," clearly indicates that Brittlebank was not yet ready to draw a definite conclusion on the subject.

Kirby (*8*) also has studied the development of the take-all disease under greenhouse and field conditions, and he recommends the adjustment of soil reaction as one of the three possible methods for protection against the disease. In the first greenhouse experiment, he conducted inoculation experiments in 5-inch pots filled with soil to which chemicals in varying quantities had been added. Very abnormal growth occurred in all cases, and a few plants reached a state of maturity only in the case of the sulphur-treated soil. No determinations of soil reaction were made, but nevertheless he endeavors to correlate the severity of the disease with an alkaline reaction. However, his figures actually show that, while the least infection did appear in the sulphur series, a higher percentage of plants were killed on the untreated soil than on the limed soil. In another greenhouse series with soil adjusted to various reactions by regulated additions of sulphuric acid or sodium hydroxide, all the plants growing in soil ranging from P_H 5.2 to P_H 8.8 were severely attacked by the fungus and ultimately killed by the disease. Soil reactions of P_H 2.3, 3.0, and 4.0 seemed to control the disease, but these soil reactions were unfavorable to the development of the host. The plants remained alive during the course of the experiment, but they never attained a height greater than a few inches. Under the condition of the experiment, therefore, Kirby concluded that no degree of active acidity which permitted normal growth and maturity of the plants controlled the disease.

Kirby (*8*) has studied the disease response to sulphur, sulphuric acid, and lime when added to the soil under field conditions in New York State. He obtained very high percentages of diseased plants in the sulphuric acid, lime, and untreated plats, and a somewhat lower but moderately high percentage in the sulphur plat. No hydrogen-ion determinations were made on the soils in question. While the addition of lime increased and that of acid decreased the percentages of badly diseased plants, it appears that no striking correlation existed between the total percentages of diseased plants and the addition of alkaline or acid substances. The disease was not effectively controlled by the additions of sulphuric acid in various proportions to the soil, and it was only partially controlled by the additions of sulphur. Moreover, in the sulphur series, the average weight of grain per plant for the healthy plants and for practically all of the diseased plants was strikingly small. Such low yields of grain on very acid soils certainly inhibit any profitable control of the disease by the applications of acid or acid-forming substances to the soil.

The results obtained by the writers show that the nutritional and physical natures of the medium, irrespective of other factors, greatly modify the influence of the hydrogen and hydroxyl ions on the growth of the fungus in pure culture. The question naturally arises as to whether soil fertility and soil type modify the influence of the soil reactions on the growth of the fungus and the occurrence of the disease in the field. This question can not be answered definitely at present, but in the light of the diverse results obtained by the writers, it will be rather surprising if variations in soil type and fertility are not found to produce similar effects. Furthermore, it appears that the results in the control of the disease under greenhouse and field conditions by the addition of acid or alkaline substances have been vari-

able and more or less unsatisfactory from the standpoint of host development. In view of these facts, therefore, it would seem that the control of take-all by the mere adjustment of soil reaction will be uncertain.

SUMMARY

In general, *Ophiobolus graminis* grew well in pure culture on solid and liquid media over a wide range of active acidity and alkalinity. The P_H range and the P_H optimum for growth were variables, depending upon the physical and chemical nature of the medium and the environmental factors.

Comparing the different solid media, the rates of growth and the total quantities of growth were more or less uniform within the favorable P_H zones. On the corresponding liquid media, the best growth occurred consistently in potato-dextrose decoction, followed in order by Czapek's solution (slightly caramelized), corn-meal decoction, and Czapek's solution (nucleic acid).

Temperature influenced the optium reaction for fungous growth in potato-dextrose decoction, but did not materially influence the P_H range through which growth occurred. The optimum temperature was not altered by variations in the hydrogen-ion concentration.

The length of the period of growth influenced the P_H optimum for fungous growth in potato-dextrose decoction but did not appreciably modify the P_H growth range. No shifts in the optimum reaction were noted for the solid media employed.

Neither diffused light nor total darkness appreciably affected the P_H optimum or the P_H range for fungous growth. However, a slightly greater tendency for a bimodal-growth curve occurred in the cultures in the dark than in those in the light.

While the hydrogen ion generally proved to be more toxic to the growth of the fungus than the hydroxyl ion, the toxicity of each was variable, depending upon the medium. It was only in a potato-dextrose decoction that the fungus was particularly sensitive to a condition of active alkalinity.

The reaction of the substrate sufficient to cause death of the fungus was several tenths of a P_H more acid or more alkaline than that sufficient to cause inhibition of growth.

Slight caramelization of Czapek's nutrient solution stimulated growth to a great degree, but severe caramelization proved toxic to the fungus. Similar relations were noted in alkaline potato-dextrose agar.

Progressive and reversible shifts in the reaction of the media were induced by the growth of the fungus. The direction and the magnitude of these shifts varied with the temperature, the duration of the growth interval, the initial reaction, and the chemical and physical natures of the media.

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EXPERIMENTAL DISTURBANCES IN THE MILK SECRETION OF THE COW [1]

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INTRODUCTION

The three major hypotheses that have been brought forth to account for the processes involved in the secretion of milk and its constituents are: (1) That the secretory cells themselves break loose, become disintegrated, and set free their contents in the alveoli; (2) that the portion of the secretory cell next to the alveoli—that is, the free end—after becoming loaded with secretory products, breaks loose from the basal portion, becomes disintegrated, and sets free its contents in the alveoli; (3) that the secretory cells discharge their secretory products into the alveoli, as do the salivary glands, without themselves becoming detached or destroyed.

The third of these hypotheses seems to the writer the most reasonable, and in the following paper experimental data are presented which support this view.

Eckles and Shaw (*3*),[3] studying the variations in the composition of milk from the individual cow, found that normally the percentage of lactose, ash, and protein content fluctuates only slightly from one milking to another, whereas the percentage of fat content is quite variable. Jackson and Rothera (*5*) found that this normally slight variability in the percentage of lactose and ash content of cow's milk could be disturbed by returning a portion of milk to the udder after it was milked dry. The percentage of lactose in the milk produced at the milking following the return of milk was greatly decreased and the percentage of ash content (soluble ash) similarly increased During the subsequent milkings both constituents gradually returned to their original condition. The freezing point of the milk produced both before and after the return of milk was practically the same, showing that there was a compensatory inverse relation between the percentage of lactose and soluble ash in the milk which tended to maintain a constant freezing point.

Owing to this very peculiar condition caused by the return of milk to the udder, a similar experiment was performed for the purpose of possibly securing an insight into the mode of milk secretion.

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[2] The author acknowledges his indebtedness to W. L. Gaines, M. J. Prucha, and J. M. Brannon for their kind assistance and suggestions during the progress of this investigation.
[3] Reference is made by number (italic) to "Literature cited," p. 885.

OUTLINE OF EXPERIMENT

The experiment consisted of seven trials and involved the use of a purebred Jersey cow milked twice a day, and in the seventh month of her lactation. In trials 1 to 4 milk was returned to a quarter of the udder after it was milked dry. In trials 5 and 6 an isotonic salt solution was injected into a quarter of the udder after it was milked dry, and in trial 7 an 8.8 per cent solution of lactose, also isotonic, was similarly injected. Each trial was divided into two periods, one preliminary to the return of milk or the injection of solutions and one subsequent to it. In all the trials each quarter of the udder was milked separately and the milk produced was weighed and sampled separately. The sample of milk from each quarter during the preliminary and subsequent periods was analyzed for the percentages of fat, lactose, and total solids. It was impossible in most cases to make a complete analysis including the determination of protein and ash; hence these two were determined by difference, and are reported as protein+ash. In trials 6 and 9, however, a complete analysis was made.

The method of returning milk or injecting solutions to a quarter of the udder after it had been milked dry was very simple and apparently did not produce any pain, for the cow showed no signs of excitement. The usual simple method of injecting a teat canula into the teat of the udder was followed. To the canula was attached a piece of rubber tubing into the other end of which the tip of a pipette was inserted, the injection thus being easily accomplished. When milk was returned the udder was washed with warm water just before milking and the milk was received in sterilized cans. The milk returned to a quarter was the same milk that had been drawn from it. The milk was returned immediately after milking, so that its temperature was practically the same as when it was drawn. When salt or lactose solutions were used, they were sterilized in an autoclave and cooled to body temperature before being injected into the udder. An examination of the milk returned showed in every case that it was practically free from bacteria.

EXPERIMENTAL RESULTS

EFFECT OF RETURNING MILK TO THE UDDER

The four trials in which milk was returned to a quarter of the udder gave similar results, all of which confirm the findings of Jackson and Rothera (*5*) with respect to the variability in the percentage of lactose content of the milk produced. The results from two of these trials are reported graphically in Figures 1 and 2. A consideration of the curves in these figures brings out the following facts with respect to the variability in the percentage content of the milk produced during both the preliminary and subsequent periods.

The percentage of lactose and the percentage of protein+ash are not extremely variable from one milking to another during the preliminary periods or under normal conditions. The percentage of fat, on the other hand, is quite variable from one milking to another under normal conditions. Of course, these percentages are for milk

taken from only one quarter of the udder. This fact may account for the wide variations in the percentage of fat content, but at the same time it would tend to emphasize the lack of variability in the

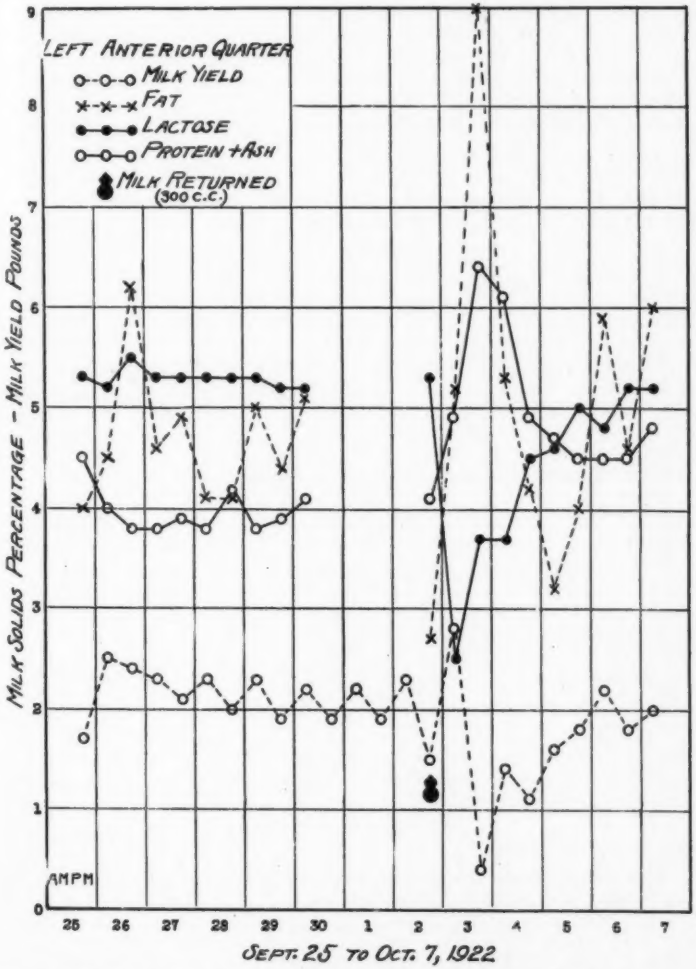
LEFT ANTERIOR QUARTER
MILK YIELD
FAT
LACTOSE
PROTEIN +ASH
MILK RETURNED (300 C.C.)
MILK SOLIDS PERCENTAGE - MILK YIELD POUNDS
AMPM
25 26 27 28 29 30 1 2 3 4 5 6 7
SEPT. 25 TO OCT. 7, 1922

FIG. 1.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the return of 300 c. c. of milk to the left anterior quarter of the udder

percentage of lactose and of protein+ash. The variation in the composition of the milk produced during the preliminary period substantiates the findings of Eckles and Shaw (*3*), although the milk comes from only one-quarter and not from the whole udder.

During the milkings subsequent to the return of milk to the udder the percentage content of the milk produced is markedly disturbed. The percentage of lactose is at first greatly decreased and then gradually returns to its normal condition. The percentage of protein+ash increases rather markedly for the first two or three milkings and then gradually returns to its normal condition. The

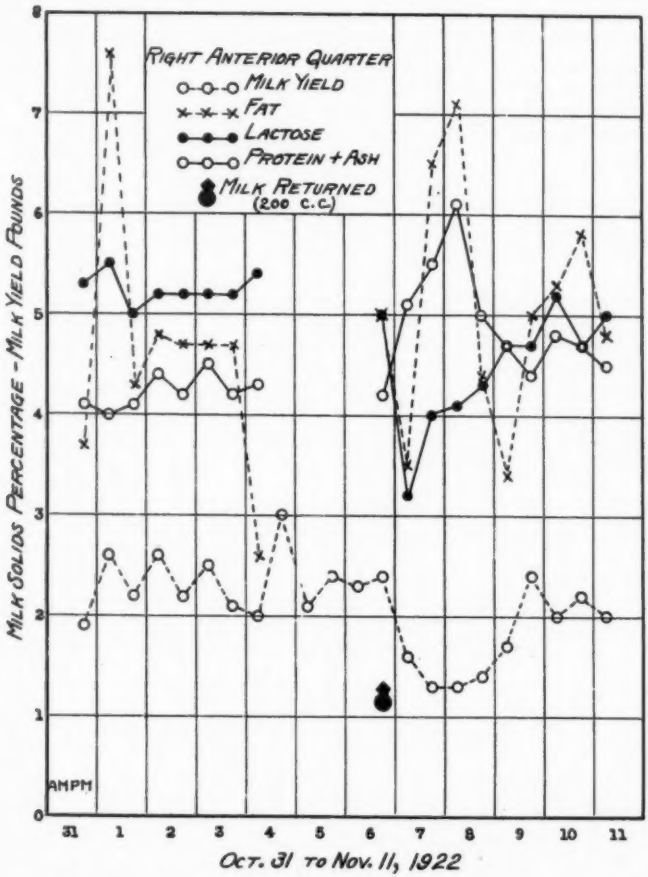
RIGHT ANTERIOR QUARTER
MILK YIELD
FAT
LACTOSE
PROTEIN + ASH
MILK RETURNED (200 C.C.)
MILK SOLIDS PERCENTAGE - MILK YIELD POUNDS
AMPM
31 1 2 3 4 5 6 7 8 9 10 11
OCT. 31 TO NOV. 11, 1922

FIG. 2.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the return of 200 c. c. of milk to the right anterior quarter of the udder

percentage of fat content increases markedly during the second or third milking and then becomes quite variable. The percentage of fat content is quite variable under normal conditions, hence the increase after the return of milk to the udder may not at first seem significant. However, this increase was always consistent, and when solutions instead of milk were injected the percentage of fat content

increased beyond any conceivable variability under normal conditions. The freezing point of the milk produced during the preliminary and subsequent periods was not determined for every sample in these trials. Those that were determined, however, indicated that it changed very little even when the percentage content was greatly disturbed.

During the first two trials the milk produced by the quarters of the udder other than the experimental quarter was also analyzed. The analysis showed that the return of milk to one quarter did not affect the composition of the milk produced by the other quarters.

Considering the variability in the percentage content of the milk produced both before and after the return of milk to the udder, it is obvious that a slight deviation in the percentage of lactose or the percentage of protein + ash is far more significant than a somewhat larger deviation in the percentage of fat.

The degree of disturbance in the percentage content of the milk produced is dependent upon the quantity of milk returned in proportion to the yield of the quarter. The yields of the quarters represented in Figures 1 and 2 are nearly equal. Three hundred c. c. of milk was returned to the quarter represented in Figure 1 and 200 c. c. was returned to the quarter represented in Figure 2. The degree of disturbance in the percentage content of the milk produced is greater in Figure 1.

EFFECT OF INJECTING SALT AND LACTOSE SOLUTIONS INTO THE UDDER

In seeking a reason for this disturbance in the composition of the milk it was decided to inject the udder with solutions instead of milk, thinking that possibly such a procedure would give some clue to the cause of the disturbance. Accordingly 300 c. c. of an isotonic salt solution, consisting of the salts found in milk (sodium and potassium chloride and sodium and potassium citrate) was injected into a quarter of the udder after it was milked dry. The results from this the fifth trial are reported graphically in Figure 3.

From a consideration of the curves in this figure it is quite surprising to see that the injection of an isotonic salt solution produced a disturbance in the milk constituents similar to that produced by the return of milk to the udder. The effect upon the percentage of fat is very pronounced, raising it greatly above any variation that could be expected under normal conditions.

Trials 6 and 7 were run together and consisted of the injection of 300 c. c. of an isotonic salt solution to one quarter and 300 c. c. of an isotonic (8.8 per cent) lactose solution to another quarter. In these trials a complete chemical analysis was made of the milk produced at the milking done at the time of the injection and during part of the subsequent milkings. The results from these two trials are reported in Tables 1 and 2.

TABLE 1.—*Effect on the yield, composition, freezing point, and specific gravity of milk produced by the injection of an isosmotic salt solution into the right posterior quarter of udder, milked twice a day, December 18 to December 27, 1922*

Milkings	Milk yield	Fat	Lactose	Protein +ash	Protein	Ash	Freezing point	Specific gravity
Preliminary milkings:	*Lbs.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*°C.*	
A. m.	2.2	5.4	4.6	[a] 4.8				1.0328
P. m.	2.3	5.2	4.8	[a] 5.3				1.0355
Milking at time of injection of 300 c. c. of isosmotic salt solution, p. m.	2.1	3.4	4.7	4.18	3.40	0.78	−0.547	1.0347
Subsequent milkings[b]	2.2	3.0	2.9	4.50	3.70	.80	−.548	1.0281
	.5	8.2	3.5	6.16	5.26	.90	−.559	1.0329
	1.7	10.0	3.7	6.34	5.50	.84	−.560	1.0326
	1.4	6.9	4.1	5.15	4.33	.82	−.545	1.0309
	1.2	3.6	4.2	4.53	3.70	.83	−.540	1.0330
	2.2	3.3	4.0	4.50	3.70	.80	−.545	1.0319
	2.2	4.4	4.2	[a] 4.6				1.0311
	1.3	3.2	4.4	[a] 4.8				1.0334
	1.9	4.3	4.3	[a] 4.9				1.0328

[a] Determined by difference.
[b] Commencing with the first one, which was done in the morning, subsequent milkings were done alternately in the evenings and mornings.

TABLE 2.—*Effect on the yield, composition, freezing point, and specific gravity of milk produced by the injection of a lactose solution into the left posterior quarter of udder, milked twice a day, December 18 to December 27, 1922*

Milkings	Milk yield	Fat	Lactose	Protein ash	Protein	Ash	Freezing point	Specific gravity
Preliminary milkings:	*Lbs.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*°C.*	
P. m.	2.1	5.2	5.0	[a] 4.5				1.0334
A. m.	1.9	4.0	5.2	[a] 4.8				1.0351
Milking at time of injection of 300 c. c. of lactose solution, p. m.	1.8	2.5	5.0	4.53	3.80	0.73	−0.549	1.0373
Subsequent milkings[b]	1.2	4.0	3.3	3.63	3.00	.63	−.550	1.0261
	.2	0.1	2.4	6.32	5.42	.90	−.550	1.0240
	1.0	12.0	2.9	7.54	6.66	.88	−.570	1.0312
	1.0	8.7	3.5	5.49	4.61	.88	−.532	1.0300
	1.3	4.9	4.0	4.38	3.56	.82	−.547	1.0320
	1.5	2.9	4.1	4.35	3.55	.80	−.542	1.0307
	2.2	3.6	4.2	[a] 4.4				1.0311
	1.2	2.9	4.4	[a] 4.5				1.0326
	1.5	3.5	4.5	[a] 4.7				1.0334

[a] Determined by difference.
[b] Commencing with the first one, which was done in the morning, subsequent milkings were done alternately in the evenings and mornings.

The variability in the percentage contents reported in Tables 1 and 2 show that with few exceptions the results from these trials are similar to those secured in the other trials. The percentage of fat content, like that in trial 5, is increased far beyond any expected deviation under normal conditions. The percentage of ash and the percentage of protein both increased after the solutions were injected into the udder. Their sum, the percentage of protein+ash, showed variability similar to that found in the other trials. On the first milking after the injection of the lactose solution the percentage of lactose was not as low as on the second milking. In all of the other trials the percentage of lactose was lowest on the first milking after the return of milk or the injection of the salt solution. This exception is due to the fact that a large quantity of lactose was injected into the quarter in the 8.8 per cent solution. The actual quantity of lactose produced at this milking was only 65.8 per cent of the quantity introduced into the quarter in the 300 c. c. of

the 8.8 per cent solution. It will also be noticed in Tables 1 and 2 that the freezing point of the milk changed only very slightly during the subsequent milkings.

EFFECT OF INJECTING DISTILLED WATER INTO THE UDDER

It was next planned to inject 300 c. c. of distilled water into a quarter of the udder after it was milked dry, but unfortunately the

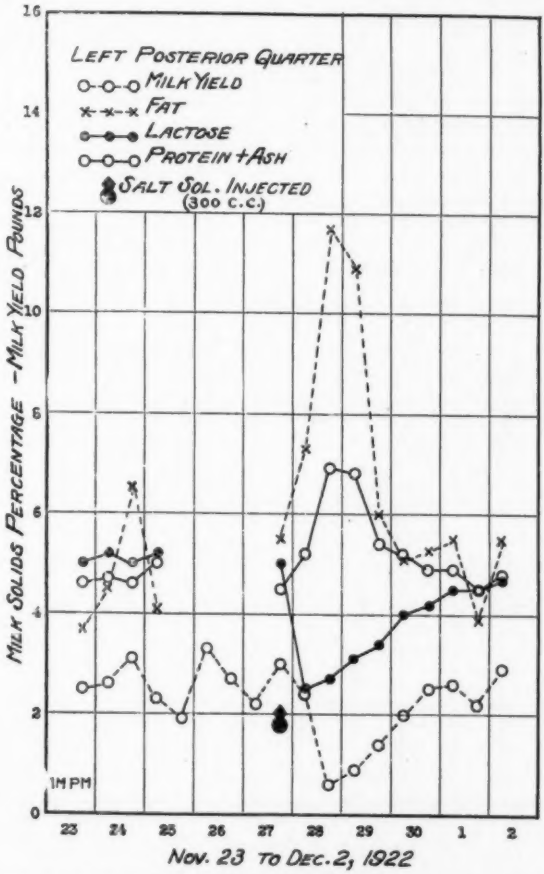

FIG. 3.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the injection of 300 c. c. of a salt solution into the left posterior quarter of the udder

experiment had to be discontinued. However, it has since been resumed, and sterile distilled water was injected into two quarters of the udder of a cow producing approximately the same quantity of milk as the cow used in the first part of the experiment. The method of procedure followed in injecting distilled water into the

udder was the same as that used in injecting salt and lactose solutions.

The results obtained in these two trials are graphically reported in Figure 4. In the milkings subsequent to the injection of 300 c. c. of distilled water only a very slight tendency was noted toward an

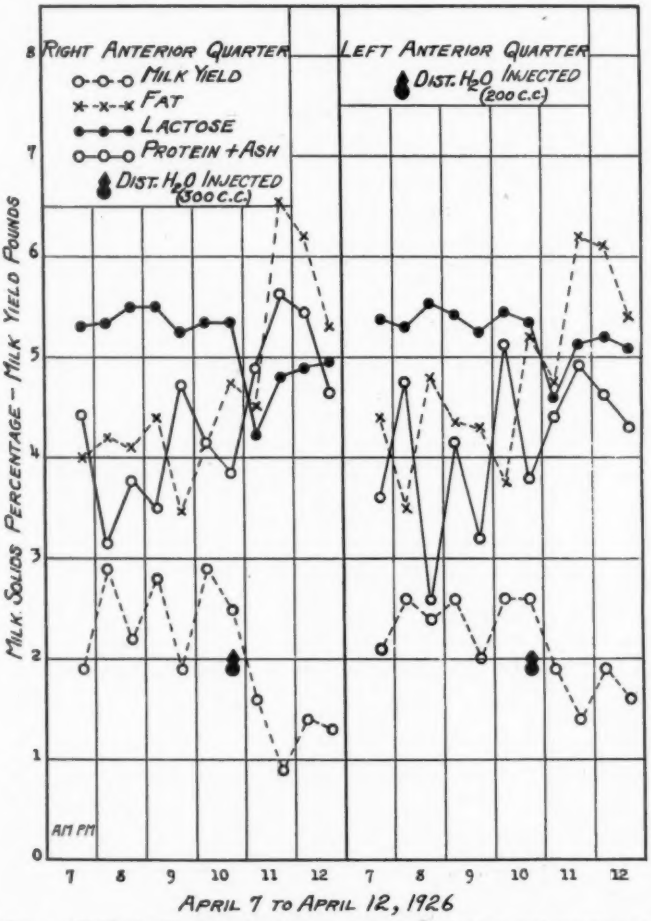

FIG. 4.—Yield of milk and percentage content of fat, lactose, and protein + ash in milkings before and after the injection of 300 c. c. of distilled water into the right anterior quarter, and 200 c. c. of distilled water into the left anterior quarter of the udder

abnormal variation in the percentage content of the milk such as was produced by returning 300 c. c. of milk or injecting salt or lactose solutions. In the milkings subsequent to the injection of 200 c. c. of distilled water there was likewise no significant abnormal variation in the percentage content of the milk. Considering the results of

these two trials, it seems as though there must be some factor other than the mere physical effect of the liquid which brings about the marked abnormal variations in the percentage content of the milk noted in the earlier experiments. This point will be further discussed later on.

EFFECT OF LEAVING MILK WITHIN THE UDDER

After the completion of the experiment described above a somewhat similar experiment was undertaken (*2*). In this test a study was made of the effect of a partial milking of the udder on the composition of the milk produced during the subsequent milkings.

The results from all of the trials in this test, wherein one-fourth, and then one-half, of the average daily evening production was left within the udder, indicate that there is very little similarity, if any, in the effect produced by leaving a portion of milk within the udder and returning a portion to the udder. The curves in Figure 5 illustrate the variations in the precentage content of the milk as a result of leaving one-half of the average daily evening production within the udder. The only percentage that seems to be affected to any extent is that of fat content.

DISCUSSION

Apparently the returning of a portion of milk to the udder after it is milked dry has an effect upon the secretive tissues of the udder altogether different from that produced by leaving a portion of milk within it. The writer can give no exact explanation for this difference. However, a description of some of the possible changes which may be taking place within the secretive tissues as a result of these manipulations of the udder may throw some light upon the subject.

A description of the processes involved in secretion is very precisely given by Bayliss (*1, p. 348*) in the following quotation:

> On the whole, it appears that the usual process of secretion is somewhat as follows: During the period of rest, the cells build up compounds which are preliminary stages of constituents of the secretion, which is afterwards set going by excitation, nervous or chemical. The formation of this material is probably a reversible reaction, so that, after a time, further production ceases, owing to accumulation of products. When the gland is excited to activity, a current of water is set flowing through the cell by some means, probably of an osmotic nature and effected by a combination of increased permeability of the outer end of the cell together with splitting up of some substance into smaller molecules. This current of water washes out into the duct the substances of the secretion already stored in the cell, sometimes after they have been further changed by a process which does not take place until the cells are excited to secretory activity. As the stored substances are lost from the cell, there will be a renewed formation to reestablish equilibrium; so that, if the activity is not too violent, there will be a balance between the amount secreted and its new formation. Continuous secretion will thus be possible without fatigue. It will be seen that, on this view, the increased production in the cell of the substances which give rise afterwards to the actual products contained in the secretion is not to be supposed to be under the control of the nervous system or other excitatory influence, but that it is a spontaneous activity of the cell itself, controlled by chemical equilibrium.

There is no reason to believe that the processes involved in the secretion of milk are different from those as described by Bayliss.

The elaboration of the milk constituents no doubt takes place in the main during the time which elapses between milkings. Vincent (*9*) cites the work of Lehmann in which a solution of sulphindi-

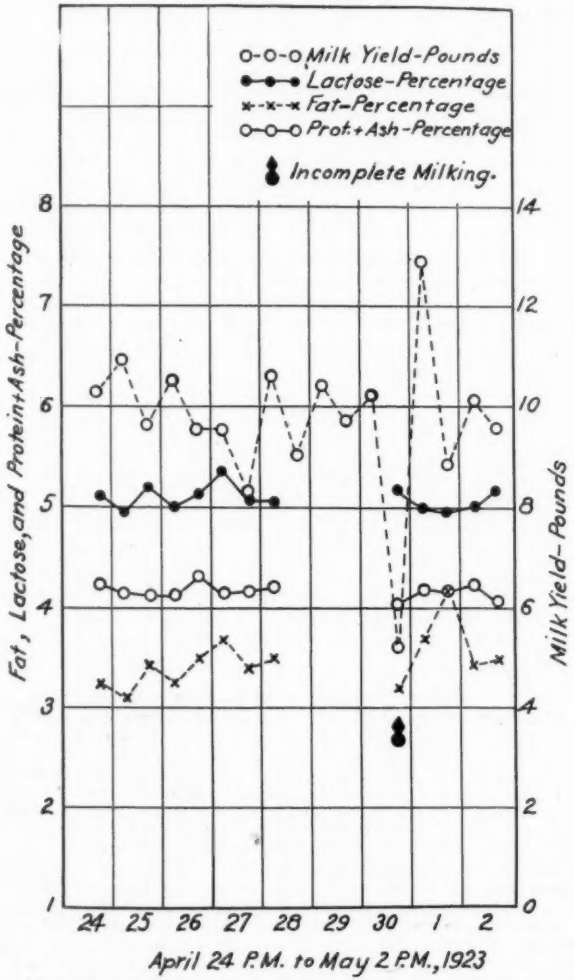

FIG. 5.—Yield of milk and percentage content of lactose, fat, and protein + ash in milk taken at complete milking and at milking where one-half of the average daily evening production was left in the udder

gotate was injected into the vein of a milk goat before milking. In the milking which followed the milk received just before the udders were emptied had a slightly bluish tinge. One hour and a half later the goat was milked again. At this time the milk was

quite blue in color, thus showing that elaboration of the milk constituents took place for the most part between the milkings.

The rate of elaboration of the milk constituents tends to follow the law of mass action, i. e., the rate is inversely proportional to the quantity of the constituents accumulated. Ragsdale et al. (*8*), working with dairy cows, have shown that, following a milking, each hour's milk production is 95 per cent of the production for the preceding hour. The percentage of total solids in the milk produced at the third hour after milking was 15.6 and at the fourteenth hour after milking it was 12.7. Here, again, we find experimental data which indicate that milk secretion follows the same general law that governs other glandular secretion as described by Bayliss.

Macallum (*7*) and Garmus (*4*) have demonstrated that the permeability of a secreting cell changes during that part of secretion which is concerned with the discharge of the secretory products stored in the cell. The membrane at the outer end of the cell next to the lumen becomes permeable, and the stored products, brought about by elaboration, are washed out into the lumen or ducts receiving the secretion. During the discharge of the secretory products there is always a greater concentration on the inside of the secretory cells than on the outside, because of the accumulation of the stored secretory products within the cells. In the case of milk secretion, the process of discharging the secretory products takes place during milking. The stored secretory products (milk constituents) are washed out into the lumen and the solution thus formed is milk. The milk is continually being drawn off during the process of milking, hence there is a continuous flow from a more concentrated solution within the cells to a less concentrated solution in the lumen. This process continues until the udder is milked dry, at which time the secretory cells have completely discharged their contents. With the complete discharge of the secretory products, the concentration within the cells is greatly lowered and at the very end of the process is lower than that of the milk which has been drawn off during milking.[4] If in case a solution of the same concentration as that of the milk drawn off were returned to the udder at this time it would work its way up into the secretive tissues, as a result of capillary attraction, and there would then be a greater concentration on the outside of the secretory cells than on the inside. The cell membrane immediately after milking (discharge of the secretory products) would still be permeable, and owing to the greater concentration of the solution on the outside of the cells, there would tend to be an absorption (diffusion into the cells) of the solutes present in the solution.

Returning to the results of the experiment (Tables 1 and 2) wherein an isotonic salt solution and an isotonic lactose solution were injected into a quarter of the udder after it was milked dry, it was found that at the milking immediately following, neither the

[4] This hypothesis as stated does not conflict with the fact that the percentage of fat content of the milk produced at the very end of a milking is much greater than the percentage of fat content of the milk produced at the very beginning of a milking. The most generally accepted explanation of this difference in the percentage of fat contents is given by Kirchner (*6*). According to this writer the fat globules, especially the larger ones, become mechanically obstructed in the milk ducts of the udder during milking and escape in large quantities in the last milk drawn.

quantity of salt nor the lactose recovered was equivalent to that introduced into the udder in the solutions. Hence, there must have been a partial absorption of both substances by the secretory cells or other cells of the gland. The same must have been true also with respect to the lactose in the milk returned.

The injection of milk or salt and lactose solutions into the udder brought about a marked abnormal variation in the percentage contents of the milk produced during the immediate subsequent milkings, but the injection of distilled water brought about no such abnormal variation. Hence it seems as though this disturbance in the percentage content of the milk is, for the most part, due to the partial absorption of the solutes present in the milk or salt and lactose solutions injected into the udder. It may be possible that this partial absorption of the solutes in the milk or solutions injected produces an abnormal condition in the secretory cells which disturbs their normal functioning. This in turn, of course, would bring about a disturbance in the whole percentage content of the milk produced during the immediate subsequent milkings.

On the other hand, when milk is left in the udder the secretory cells in the secretive tissue never completely discharge their products, and therefore never reach the same condition as when the udder is milked dry. Although there is a film of milk in contact with the cells in this condition, it does not bear the same relation to the cells as it would if the cells had completely discharged their products. The concentration within the cells would still be greater than that of the milk present in the lumen. Hence if there were to be any diffusion of solutes, which no doubt takes place, it would be out of the cells and not into them. When the act of milking ceases the stimulus producing the discharge of the cell products also soon ceases and the whole process is brought to a halt. If there is no absorption of solutes, then there should also be no disturbance in the composition of the milk produced during the subsequent milkings. This reasoning is supported by the fact that there is apparently very little disturbance in the composition of the milk when even one-half of the production is left in the udder, whereas there is a very marked disturbance when only a small fraction of the production is returned after the udder has been milked dry.

SUMMARY OF EXPERIMENTS

A portion of milk was returned to the udder of a cow after it had been milked dry. The effects upon the composition of the milk produced during subsequent milkings were as follows: (1) the percentage of lactose markedly decreased on the first subsequent milking and then gradually returned to its original condition; (2) the percentages of protein and ash increased during the first two or three milkings and then returned to their original condition; (3) the percentage of fat increased very markedly during the first two or three milkings and then became quite variable.

Three hundred cubic centimeters of an isotonic salt solution, 300 c. c. of an isotonic lactose solution, and 300 c. c. of distilled water were, one at a time, injected into the udder after it was milked dry.

The isotonic salt and lactose solutions produced an effect similar to the returning of milk to the udder. The distilled water produced very little disturbance in the composition of the milk.

In order to determine the effect of a partial milking on the composition of the milk subsequently produced, one-fourth, and later one-half, of the average daily evening production was left within the udder. The only percentage that showed any great modification was that of fat content.

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THE EFFECT OF FEEDING CELLULOSE ON THE PULSE RATE OF STEERS[1]

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INTRODUCTION

Benedict and Ritzman,[3] in their study of undernutrition in steers, observed an almost immediate drop in pulse rate following curtailment of ration, and a very marked increase in pulse rate when their steers were placed on a fattening ration. They concluded that "the pulse rate and metabolism with steers are very closely in agreement," and inferred "that with steers, when there is a change in pulse rate with the same animal, there is a simultaneous change in metabolism which is roughly proportional to this change in the pulse rate." In discussing pulse rates, they stated that "the pulse rate or, more properly speaking, the heart rate, is an index of the relative amount of circulatory activity, and as the blood carries oxygen to the tissues and brings back carbon dioxide to the lungs, the heart rate may be taken as a crude, approximate index of relative metabolic intensity."

In the course of an investigation to determine the effect of crude fiber (paper pulp) on the digestibility of alfalfa by steers, pulse rate data were obtained for the purpose of getting an indication of the metabolic levels of the animals when consuming a ration of alfalfa and when consuming rations of alfalfa and paper pulp in different proportions.[4] The results were so striking that it seemed desirable to bring them to the attention of other workers in animal nutrition.

EXPERIMENTAL PROCEDURE

In the investigation referred to in the preceding paragraph five "long yearling" steers [5] were fed for a period of 226 days, and during that time a series of nine digestion trials was conducted. Four of the five steers were used in each of the first two trials and three of them were used in each of the last seven. The digestion trials were 10 days in length and the intervals between them were: (a) 11 days between trials on the same ration, and (b) 18 days between trials on different rations. It was during these nine digestion trials that the pulse rate data herein recorded were obtained.

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[1] Received for publication, July 6, 1926; issued, November, 1926.

[2] Now associate biological chemist, Division of Animal Husbandry, Bureau of Animal Industry, United States Department of Agriculture.

[3] BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

[4] The complete data for this investigation are being published elsewhere. See TITUS, H. W. THE MUTUAL INFLUENCE OF THE PROPORTION OF THE SEVERAL NUTRIENTS, IN FEEDS, ON THEIR DIGESTIBILITY: I CRUDE FIBER—THE DIGESTIBILITY OF RATIONS CONTAINING VARYING AMOUNTS OF ALFALFA AND PAPER PULP. N. Mex. Agr. Expt. Sta. Bul. 153. (In press.)

[5] Their average initial live weight was 586 pounds and their average final live weight was 774 pounds.

The weight of feed given daily to each steer was 12 pounds [6], one half of this being fed at 5 p. m. and the other half at 6.30 p. m.[7] One steer received alfalfa and two received mixtures of alfalfa and paper pulp, in different proportions, throughout the investigation; the other two steers received alfalfa part of the time and mixtures of alfalfa and paper pulp the rest of the time (Table 3).

The steers were watered at 3 p. m. and 10 a. m. Pulse rates were taken during the half-hour period preceding each watering; the technic of Benedict and Ritzman was employed.[8]

COMPOSITION OF THE COMPONENTS OF THE RATIONS, AND OF THE RATIONS

The composition of the dry matter of the alfalfa and paper pulp used in compounding the rations is given in Table 1. The composition (as fed) of the feed used in the nine digestion trials is given in Table 2. Five different rations (four of them definite mixtures of alfalfa and paper pulp) were fed. The percentage of each of the two components in each of the rations was as follows:

	Ration No. 1	Ration No. 2	Ration No. 3	Ration No. 4	Ration No. 5
Alfalfa (per cent)	100	85	70	55	40
Paper pulp (per cent)	0	15	30	45	60

The first cutting of alfalfa of very good quality was used throughout the investigation. The paper pulp employed was a high-grade whole rag commercial product. The main constituent of the paper pulp was cellulose which (determined as crude fiber) made up approximately 87 per cent of the dry matter.

TABLE 1.—*Composition of the dry matter of the alfalfa and paper pulp*

ALFALFA

Statistical constants	Ash	Protein	Nonprotein	Crude fiber	Ether extract	Nitrogen-free extract	Total nitrogen	Protein nitrogen
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mean [a]	10.97	14.03	2.12	32.78	1.62	38.48	2.694	2.244
Probable error of mean	±.03	±.06	±.02	±.10	±.01	±.08	±.009	±.009
Standard deviation	±.19	±.40	±.14	±.70	±.09	±.55	±.064	±.065
Coefficient of variation	1.76	2.88	6.74	2.15	5.49	1.42	2.39	2.88

PAPER PULP

Mean [b]	0.94	0.19	Trace.	87.12	0.10	11.64	0.030	0.028
Probable error of mean	±.03	±.02	.....	±.25	±.01	±.25	±.002	±.002
Standard deviation	±.20	±.09	.....	±1.46	±.04	±1.44	±.009	±.010
Coefficient of variation	21.08	46.02	.....	1.67	35.87	12.37	29.11	46.02

[a] Computed from the results of 22 separate analyses; the average moisture content of the 22 samples was 8.44 per cent.

[b] Computed from the results of 15 separate analyses; the average moisture content of the 15 samples was 5.67 per cent.

[6] Except in the case of one steer (No. 44), which was fed 9.5 pounds, 10.5 pounds, and 9.5 pounds per day during the last three digestion trials (Nos. 7, 8, and 9), respectively.

[7] The experimental day began at 12 m.

[8] BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

TABLE 2.—*Composition of the feed used in the nine digestion trials* [a]

Ration No.	Digestion trial No.	Moisture	Ash	Protein	Nonprotein	Crude fiber	Ether extract	Nitrogen-free extract	Total nitrogen	Protein nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	1	8.33	10.25	13.64	1.84	29.68	1.59	34.67	2.573	2.182
	2	8.85	9.80	12.71	2.02	29.84	1.54	35.24	2.464	2.034
2	3	8.32	8.64	10.63	1.63	38.06	1.26	31.46	2.047	1.701
	4	9.76	8.17	10.26	1.55	38.52	1.17	30.57	1.971	1.642
3	5	8.32	6.99	8.38	1.55	46.61	.92	27.23	1.672	1.341
	6	7.15	7.05	8.55	1.38	47.07	.96	27.84	1.660	1.368
4	7	6.57	5.90	6.80	1.05	55.50	.67	23.51	1.310	1.088
	8	5.44	6.01	7.21	1.10	55.86	.67	23.71	1.386	1.152
5	9	4.87	4.71	5.06	.79	63.90	.45	20.22	.977	.810

[a] As fed.

THE PULSE RATES

The pulse rates are given in Table 3; these are the averages of the afternoon (2.30 p. m.) and morning (9.30 a. m.) pulse rates. The morning pulse rates were almost invariably greater than the afternoon pulse rates; the former averaged 2.8 beats per minute more than the latter. The individual average differences were as follows: Steer No. 41, 3.1; steer No. 42, 5.1; steer, No. 43, 1.6; steer No. 44, 2.2; steer No. 45, 1.9; average, 2.8.

TABLE 3.—*The feed and the pulse rates of the steers*

Digestion trial No.	Feed given	Average pulse rates [a]				
		Steer No. 41	Steer No. 42	Steer No. 43	Steer No. 44	Steer No. 45
		Beats per minute	*Beats per minute*	*Beats per minute*	*Beats per minute*	*Beats per minute*
1	Ration No. 1	(b)	**59.3**	**54.0**	[c] 63.7	**56.7**
2	...do	(b)	**58.0**	**52.1**	[d] 67.9	**54.4**
3	Ration No. 2		**56.1**	**53.8**		**58.2**
4	...do		**57.8**	**53.8**		**58.9**
5	Ration No. 3		**63.9**	**56.1**		**62.6**
6	...do		**58.4**	**58.2**		**66.1**
7	Ration No. 4		**65.0**	**65.8**	[e] 60.0	
8	...do		**62.6**	**64.7**	[f] 61.6	
9	Ration No. 5		**64.1**	66.1	[g] 60.9	
(h)						
3	Alfalfa [i]	76.1			53.2	
4	...do	70.0			54.0	
5	...do	69.2			52.5	
6	...do	69.1			53.4	
7	...do	64.9				[e] 58.4
8	...do	58.7				52.7
9	...do	59.8				54.5

[a] The pulse rates riven in bold-face figures are the ones around which the discussion centers.
[b] Pulse rates were not taken. Steer No. 41 was not used in any of the digestion trials.
[c] This steer had a higher temperature (rectal) than the others during this digestion trial and went "off feed" for several days following this trial.
[d] This steer had a higher temperature (rectal) than the other steers during this digestion trial and did not eat all the feed offered it.
[e] A week after the end of digestion trial No. 6 steer No. 45 had an impacted intestine. Steer No. 44 was used in place of steer No. 45 in the last three digestion trials. Steer No. 44 received only 9.5 pounds of feed per day during digestion trial No. 7.
[f] Received only 10.5 pounds of feed per day.
[g] Received only 9.5 pounds of feed per day.
[h] Pulse rates given below the dotted line are for the steers *not* used in the digestion trials.
[i] Essentially ration No. 1.

DISCUSSION

The data given in Table 3 show that as rations containing increasing quantities of paper pulp (cellulose) were fed, there was a marked tendency for the pulse rate of the steers to increase. They also show that when (as in the case of steer No. 45) a ration consisting solely of alfalfa was fed in place of one containing paper pulp, the pulse rate dropped immediately; and that when (as in the case of steer No. 44, during the last seven digestion trials) [9] a ration containing paper pulp was fed in place of one consisting solely of alfalfa, the pulse rate immediately increased, even though the weight of the ration containing paper pulp was less [10] than that containing nothing but alfalfa.

In the case of steer No. 41, which received a ration of alfalfa throughout the investigation, a steady decrease in pulse rate was observed. This animal, unlike the others, was rather nervous and did not become accustomed to its pulse rate being taken until after it had been under observation for several weeks. The drop in pulse rate between digestion trials No. 3 and No. 4 was probably due, at least in part, to the fact that the steer had become accustomed to having its pulse rate taken. A possible explanation of the decrease in pulse rate after the fourth digestion trial is that the fixed weight of alfalfa, which was fed throughout, furnished less and less energy, over and above that required for maintainance, as the weight of the animal increased.

This decrease in the pulse rate of a steer on a fixed ration of alfalfa makes the increase in the pulse rate of the steers receiving rations containing increasing quantities of paper pulp of even greater significance.

If the pulse rate is an index of the relative metabolic intensity, or metabolic level, it would seem as if a given weight of the paper pulp were more efficient than an equal weight of alfalfa in supplying energy. The results of the digestion trials showed that the dry matter of the paper pulp had an apparent digestibility of approximately 77.56 per cent and that the dry matter of the alfalfa had an average apparent digestibility of 59.72 per cent, the latter coefficient of digestibility being to the former as 1 is to 1.3. As will later be shown, this ratio is nearly the same as the ratio of the (calculated) pulse rate which would result from feeding paper pulp alone to that found when alfalfa is fed alone.

In order to secure a numerical measure of the effect produced by paper pulp on the pulse rate, the following equation was used:

$$P = k\,(A + mB) \qquad (1)$$

in which:

P = pulse rate observed when a given ration is fed.
A = amount of alfalfa in the ration (expressed as a fraction of the total ration).
B = amount of paper pulp in the ration (expressed as a fraction of the total ration). (Note: A + B must equal unity.)
k = pulse rate which would be observed if the ration consisted solely of alfalfa.
m = ratio of the to-be-calculated pulse rate resulting from feeding paper pulp alone to that found when alfalfa is fed alone.

[9] The pulse rates of steer No. 44 were abnormally high, as were the rectal temperatures during the first two digestion trials, and for that reason can not be compared with the pulse rates obtained during the seven digestion trials which followed. See footnotes *c* and *d* of Table 3.

[10] See Table 3, footnotes *e*, *f*, and *g*.

For the sake of convenience, equation (1) may be written in the form:

$$P = kA + kmB \quad (2)$$

or:

$$P = kA + nB \quad (3)$$

in which, $n = km$ (or $m = n/k$), n being the calculated pulse resulting from the feeding of a ration consisting solely of paper pulp.

We may now determine the values of k and n by substituting in equation (3) the values of P, A, and B and solving the resulting equations by the method of least squares.

On treating the pulse rates, given in bold-face figures in Table 3, in the manner outlined above, we get the following values for k, n, and m:

	k	n	m
Steer No. 42	57. 33	69. 52	1. 21
Steer No. 43	51. 51	76. 73	1. 49
Steer No. 45	55. 08	83. 53	1. 52
Average (weighted)	55. 05	73. 44	1. 33

The values of m for steers Nos. 43 and 45 agree quite well; the value for steer No. 42 is much lower than it is for the other steers. Incidentally, the difference between the morning and the afternoon pulse rate of steer No. 42 was greater than that of any of the other steers and, in general, the pulse rate of this steer showed the greatest fluctuations. This may account for the low value of m obtained with steer No. 42. In view of the fact that the conditions under which the pulse rates were taken were not rigidly controlled, it seemed best to treat the pulse rates of these three steers as though they were for one animal, and in this way secure an average value of m. This average value of m was found to be 1.33, which is very nearly the same as the value of the ratio of the coefficient of apparent digestibility of alfalfa to that of paper pulp. The agreement between these two ratios seems to indicate that the digestible portion of paper pulp is at least equivalent to the digestible portion of alfalfa as a source of energy.

Although Benedict and Ritzman [11] considered the pulse rate as a crude, approximate index of the metabolic intensity, it may be that if a highly refined technic were worked out for securing an average pulse rate over an extended period of time, this pulse rate would be a rather accurate index of the metabolic intensity and of great worth in comparing the nutritive values of feeding stuffs.

CONCLUSION

Cellulose (in the form of paper pulp), when substituted for an equal weight of alfalfa, in a ration consisting solely of alfalfa, tends to cause an increase in pulse rate, which is proportional to the amount substituted.

[11] BENEDICT, F. G., and RITZMAN, E. G. UNDERNUTRITION IN STEERS: ITS RELATION TO METABOLISM, DIGESTION, AND SUBSEQUENT REALIMENTATION. 333 p., illus. Washington, D. C. 1923. (Carnegie Inst. Wash. Pub. 324.)

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